A study of self-emulsifying oil/surfactant mixtures.

Pouton, Colin William

A STUDY OF SELF-EMULSIFYING OIL/SURFACTANT MIXTURES

A THESIS

presented by

COLIN WILLIAM POUTON

for the degree of

DOCTOR OF PHILOSOPHY

in the

UNIVERSITY OF LONDON

Department of Pharmacy,
Chelsea College,
University of London,
Manresa Road,
London SW3

January, 1982

ABSTRACT

A range of oil/non-ionic surfactant mixtures were allowed to 'self-emulsify' in aqueous media under conditions of controlled low agitation. In each case the ease of emulsification was assessed by measuring the speed at which emulsification took place and by determining the mean particle sizes of resultant emulsions.

Ease of emulsification was not related to densities or viscosities of oil/surfactant mixtures but was closely related to phase behaviour of oil/surfactant/water mixtures at equilibrium prior to emulsification. Good emulsification took place when corresponding phase behaviour was close to that of a critical region at which phase inversion would occur. The presence of liquid crystalline 'surfactant phase' in this locality was also of critical importance.

The effects of the following factors on the ease of 'self-emulsification' were examined; the self-emulsifying mixture composition (surfactant concentration, surfactant composition, oil identity, moisture content and the presence of dissolved substances), the aqueous phase composition and the temperature. The results were correlated with corresponding changes in phase behaviour at equilibrium.

The biological absorption of benzoic acid from various formulations administered orally was measured using six volunteers. Variation between formulations was not significantly higher than variation within formulation

groups suggesting that the rate and extent of benzoic acid absorption was not affected significantly by formulation in a 'self-emulsifying' vehicle.

ACKNOWLEDGEMENTS

I owe thanks to a large number of people.

- To R.P. Scherer Inc. for funding the project and to the staff of R.P. Scherer Ltd., particularly Mr. R. Collins and Mr. N. Grainger for their encouragement and co-operation.

- To Coulter Electronics for the loan of a Coulter Nanosizer.

- To Mr. B.W. Burt for his helpful advice and supervision during the writing of the Thesis.

- To Dr. M.J. Groves for conceiving the project and supervising its initial stages.

- To Mr. V.H. Dawes and Dr. N. Pilpel for helpful discussion throughout my studentship.

- To all the staff and students of Chelsea College, particularly those working at the Hammersmith Annexe, for their friendly, good-humoured approach to work. In this connection I am indebted to 'The Ship's Crew', particularly 'The Ship's Cat', for technical excellence and unrelenting hinderance.

Finally to Dominique for her constant warmth and encouragement.

To my parents.

CONTENTS

	Page number
ABSTRACT	2
ACKNOWLEDGEMENTS	4
DEDICATION	5
CONTENTS	6
LIST OF FIGURES	9
LIST OF TABLES	13
LIST OF PLATES	15
CHAPTER ONE. A GENERAL INTRODUCTION	
1.1 Origin and aims of the present study	17
1.2 Selection of materials	20
1.3 Scope of physical research	22
1.4 Biopharmaceutical considerations and scope of present study	24
CHAPTER TWO. EMULSIFICATION AND NON-IONIC SURFACTANTS	
2.1 Conventional emulsification	30
2.2 Model studies of emulsification	32
2.3 Spontaneous emulsification	37
2.4 Some properties of non-ionic surfactants	42
CHAPTER THREE. MATERIALS AND METHODS	
3.1 Materials	53
3.1.1 Water	53
3.1.2 Lipophilic materials	53
3.1.3 Amphiphilic materials	55
3.1.4 Other materials	57
3.2 Physical methods	59
3.2.1 Equilibrium phase behaviour	59
3.2.2 Preparation of emulsifiable mixtures	59
3.2.3 Viscosities	59
3.2.4 Densities	60
3.2.5 Physical properties of self-emulsified systems	60

Page number

3.2.5.1 Comparative assessment of emulsification rate 61
3.2.5.2 Assessment of emulsion quality 62
3.2.5.2.1 Determination of mean particle size using a Coulter Nanosizer 64
3.2.5.2.2 Examination of emulsion stability using a Coulter Nanosizer 65
3.2.5.2.3 Microscopy 65
3.2.6 Physical properties of homogenised systems 65
3.2.7 Assessment of affinity of Tween 85 for aqueous phases 65
3.2.8 Photomicroscopic study of the emulsification process 67
3.3 Biopharmaceutical Methods 68
3.3.1 Dietary restrictions 68
3.3.2 Fluid intake 68
3.3.3 Urine samples 69
3.3.4 Formulations 69

CHAPTER FOUR. RESULTS AND DISCUSSION
4.1 The behaviour of M812/T85/S85 mixtures in distilled water 72
4.1.1 Phase behaviour, viscosities and densities of oil/surfactant mixtures at equilibrium 72
4.1.2 Phase behaviour of hydrous mixtures at equilibrium 81
4.1.3 Emulsification of M812/T85/S85 mixtures 95
4.1.3.1 M812/T85 mixtures 95
4.1.3.2 M812/T85/S85 mixtures 118
4.1.3.3 Effect of water content of original oil/surfactant mixtures on self-emulsification 126
4.1.3.4 Effect of temperature on self-emulsification 133
4.2 The effect of oil identity on emulsification of oil/T85 mixtures in distilled water 142
4.3 A comparison of physical properties of alternative Tween surfactants 160
4.4 The effect of continuous phase composition on self-emulsification of M812/T85/S85 mixtures 170
4.5 The effect of benzoic acid dissolved within M812/T85 mixtures on self-emulsification 187

Page number

4.6 The importance of conditions during emulsification to subsequent emulsion quality 197

4.7 Biological absorption of benzoic acid from formulations administered orally 201

CHAPTER FIVE. SUMMARY AND CONCLUSIONS 210

APPENDICES 1. Description of the apparatus used for comparison of rate of self-emulsification 220

2. Development of an assay method for biopharmaceutical experiments 231

REFERENCES 240

LIST OF FIGURES

		Page number
2.1.	The break-up of liquid jets.	34
2.2.	Zig-zag and meander configurations of polyoxyethylene	44
2.3.	Phase diagram of water and the surfactant $C_9H_{19}.C_6H_4.O.(CH_2CH_2O)_{9.2}.H$.	46
2.4.	Phase diagram of water and the surfactant $C_{10}H_{21}.O.(CH_2CH_2O)_3.H$.	46
2.5.	The effect of temperature on the volume fractions of water and oil and the interfacial tension between these phases.	48
2.6.	The phase diagram of water and cylcohexane in the presence of 5%w/w polyoxyethylene - (8.6) - nonylphenylether as a function of temperature.	48
3.1.	General shape of curve for evaluation of emulsification rate.	63
4.1.	Phase diagram of the industrial grade materials Miglyol 812, Tween 85 and Span 85 at 25°C.	73
4.2.	Viscosities of Miglyol 812/surfactant mixtures.	75
4.3.	Densities of Miglyol 812/surfactant mixtures.	77
4.4.	Effect of moisture content on viscosities and densities of M812/T85 mixtures at 25°C.	78
4.5.	Effect of moisture content on viscosities and density of POE-(20)-S-trioleate at 25°C.	80
4.6.	Phase diagram of M812, POE-(20)-S-trioleate and water at 25°C.	83
4.7.	Phase behaviour of some M812, POE-(20)-S-trioleate, S-trioleate and water mixtures at 25°C.	85
4.8.	Phase diagram of water and POE-(20)-S-trioleate.	86
4.9.	Phase diagram of water and the mixture 80%w/w POE-(20)-S-trioleate and 20%M812	86
4.10.	Phase diagram of water and the mixture 60%w/w POE-(20)-S-trioleate and 40%M812.	88
4.11.	Phase diagram of water and the mixture 50%w/w POE-(20)-S-trioleate and 50%M812.	88
4.12.	Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50%M812 (expanded scale).	92

Page number

4.13. Phase diagram of water and the mixture 40% POE-(20)-S-trioleate and 60% M812. 92

4.14. Phase diagram of water and the mixture 30% POE-(20)-S-trioleate and 70% M812. 93

4.15. Phase diagram of water and the mixture 20% POE-(20)-S-trioleate and 80% M812. 93

4.16. Phase diagram of water and the mixture 15% POE-(20)-S-trioleate and 85% M812. 94

4.17. Emulsification times of M812/T85 mixtures in distilled water at 25°C. 97

4.18. Mean particle sizes of self-emulsified and homogenised emulsions of M812/T85 mixtures in distilled water at 25°C. 100

4.19. Guide to the efficiency of self-emulsification of M812/T85/S85 mixtures in distilled water at 25°C. 119

4.20. Self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C. 121

4.21. Self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C. 124

4.22. Effect of water content of mixtures on densities and viscosities at 25°C. 127

4.23. Effect of water content of original mixtures on emulsification time. 129

4.24. Effect of temperature on mean particle size of emulsions formed by self-emulsification of 70%M812/30%T85 in distilled water. 136

4.25. Self-emulsification times of M812/T85/S85 mixtures in distilled water at 37°C. 139

4.26. Phase behaviour of oil/T85 mixtures at 25°C. 143

4.27. Viscosities of M812/T85 and M840/T85 mixtures at 25°C. 145

4.28. Viscosities of oil/T85 mixtures at 25°C. 146

4.29. Densities of oil/T85 mixtures at 25°C. 147

4.30. Self-emulsification times of M840/T85 mixtures in distilled water at 25°C. 149

4.31. Mean particle sizes of self-emulsified and homogenised emulsions of M840/T85 mixtures in distilled water at 25°C. 150

4.32. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% octane. 153

4.33. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% octanoic acid. 153

4.34. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% octanol. 154

Page number

4.35. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% glyceryl trioleate. 154

4.36. Phase behaviour of oil/T81 and oil/T80 mixtures at 25°C. 161

4.37. Phase behaviour of Miglyol/T80/S85 mixtures at 25°C. 163

4.38. Phase diagram of water and POE-(20)-S-mono-oleate. 164

4.39. Self-emulsification of M840/T80 mixtures in distilled water at 25°C. 166

4.40. Self-emulsification of M812/T80 mixtures in distilled water at 25°C. 167

4.41. Self-emulsification of some M840/T80/S85 mixtures in distilled water at 25°C. 168

4.42. Phase diagram of 10^{-1}M HCl and the mixture 30% POE-(20)-S-trioleate and 70% M812. 175

4.43. Self-emulsification of 70%M812/30%T85 in the presence of ions at 25°C. 177

4.44. Self-emulsification of some M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C. 182

4.45. Self-emulsification of some M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C. 183

4.46. Effect of solubilized benzoic acid on self-emulsification of 70%M812/30%T85 at 25°C. 188

4.47. Effect of solubilized benzoic acid on mean particle size of emulsions formed by self-emulsification at 25°C. 189

4.48. Phase diagram of water and the mixture 5% benzoic acid in 30% POE-(20)-S-trioleate/ 70% M812. 191

4.49. Effect of water concentration on viscosities and densities of emulsifiable mixtures in the presence of benzoic acid (at 25°C). 193

4.50. Typical experimental plot of appearance of hippuric acid in urine following oral administration of benzoic acid. 203

A.1.1 Construction of the apparatus used for self-emulsification and comparison of emulsification rate. 221

A.1.2 Cell and nephelometer in vertical cross-section. 221

A.1.3 Nephelometer in horizontal cross-section. 222

Page number

A.1.4 Schematic diagram of the amplifier circuit. 222
A.1.5 Slope $\delta D_s/\delta N$ versus latex volume^2 (v^2). 225
A.1.6 Slope $\delta D_s/\delta C$ versus latex diameter. 227
A.1.7 Effect of injection volume on Rel I_{100}. 229

LIST OF TABLES

		Page number
4.1.	Rate of emulsification of M812/T85 mixtures in distilled water at 25°C.	96
4.2.	Assessment of quality of self-emulsified and homogenised emulsions of M812/T85 mixtures in distilled water at 25°C.	99
4.3.	Effect of water concentration in original mixtures on quality of emulsions formed by self-emulsification.	130
4.4.	Effect of temperature on rate of emulsification of 70%M812/30%T85 in distilled water.	135
4.5.	Quality of emulsions formed by self-emulsification of glyceryl trioleate/T85 mixtures in distilled water at 25°C.	157
4.6.	Quality of homogenised emulsions of carboxylic acid/T85 mixtures prepared in distilled water at 25°C.	159
4.7.	Self-emulsification of 70%M812/30%T85 in hydrochloric acid solutions at 25°C.	173
4.8.	Emulsification times of M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C.	180
4.9.	Quality of emulsions formed by self-emulsification of M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C.	181
4.10.	Effect of hydrochloric acid concentration on particle size of homogenised emulsions of 70%M812/30%T85 at 25°C.	185
4.11.	Light scattering of homogenised T85 dispersions (10^{-3} to 10^{-1}% w/w) in the presence of ions.	186
4.12.	Effect of water content (of original mixtures) on emulsification rate of mixtures containing benzoic acid.	194
4.13.	Effect of water content (of original mixtures) on quality of emulsions formed by self-emulsification of mixtures containing benzoic acid.	195
4.14.	Effect of storage on quality of emulsions formed by self-emulsification and homogenisation of M840/T85 mixtures in distilled water at 25°C.	198
4.15.	Effect of storage at 25°C on quality of emulsions formed by self-emulsification of 70%M812/30%T85 at various temperatures.	200

Page number

4.16-4.20. Cumulative appearance of hippuric acid in urine resulting from oral administration of benzoic acid in various formulations.

4.16. Formulation 1 powdered drug. 204

4.17. Formulation 2 non-emulsifying oil solution. 205

4.18. Formulation 3 self-emulsifying formulation. 206

4.19. Formulation 4 poorly emulsifying formulation. 207

4.20. Formulation 5. 208

4.21. Analysis of variance of ET_{50} for three formulation groups using six subjects. 209

A.1.1. Relative light scattering of polystyrene latex dispersions. 226

A.2.1. Effect of moving phase composition on retention of hippuric and benzoic acids. 233

A.2.2. Retention study of a series of benzoates. 235

A.2.3. Effect of moving phase compositions on retention of hippuric, p-OH-benzoic and benzoic acids. 237

A.2.4. Calibration of assay for hippuric and benzoic acids using p-OH-benzoic acid as a standard. 238

A.2.5. Assay for hippuric and benzoic acids in urine ('spiked urine' test). 239

LIST OF PLATES

		Page number
1-3.	Appearance of typical emulsions formed by Type A,B and C mixtures in distilled water at 25°C.	
	1. Type A	104
	2. Type B	104
	3. Type C	104
4-7.	Interfacial disruption caused by penetration of distilled water into M812/T85 mixtures at 25°C.	
	4. 85%M812/15%T85 mixture.	113
	5. 80%M812/20%T85 mixture.	113
	6. 70%M812/30%T85 mixture.	114
	7. 50%M812/50%T85 mixture.	114
8.	Interaction between the 20%M812/80%T85 mixture and distilled water at 25°C.	116
9-11.	Appearance of the oil/water interface after cessation of emulsification (M812/T85 mixtures in distilled water at 25°C).	
	9. 80%M812/20%T85 mixture.	116
	10. 70%M812/30%T85 mixture.	117
	11. 50%M812/50%T85 mixture.	117
12-14.	Emulsions formed by self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C.	
	12. 70%M812/25%T85/5%S85 mixture.	123
	13. 70%M812/22.5%T85/7.5%S85 mixture.	123
	14. 70%M812/15%T85/15%S85 mixture.	123
15-17.	The effect of hydrochloric acid dissolved within the aqueous phase on quality of emulsions formed by self-emulsification of 70%M812/25%T85/5%S85 at 25°C.	
	15. Distilled water.	178
	16. 10^{-3}M HCl.	178
	17. 10^{-1}M HCl.	178

CHAPTER ONE

A GENERAL INTRODUCTION

1.1. ORIGIN AND AIMS OF THE PRESENT STUDY

It is widely accepted that the rate of gastro-intestinal absorption of many drugs from solid dosage forms may be limited by the dissolution process[1-4]. Indeed modification of dissolution rate may be of value in the formulation of 'slow-release' products[5]. However poor dissolution of lipophilic drugs may result in undesirable incomplete or variable absorption leading to vagaries in theraputic efficacy[6].For such drugs absorption may be improved if the dissolution step is by-passed[7]. Presentation of lipophiles in oil solution may enhance absorption although it is generally accepted that the drug must diffuse into the aqueous gastro-intestinal contents before diffusing across the mucosal membrane. The mechanism of systemic absorption from oil solutions is obscure. Some authors have reported that the mere presence of oil in the gastro-intestinal tract has enhanced absorption of lipophiles[8-11]. In other studies certain drugs formulated as suspensions in oil were more efficiently absorbed than they were from corresponding aqueous or solid dispersions[12-15]. One might expect that if a solution of a drug in oil was emulsified in water the resulting increase in interfacial area would enhance absorption by increasing the rate of diffusion from oil to aqueous phase. Some papers have suggested that this can occur in practice when drug is dissolved or suspended in oil[15-16]. Several other authors have described increases in absorption of lipophiles from emulsion formulations[17-22]. The mechanism of enhanced absorption

from emulsion formulations is as uncertain as that of absorption from oil solutions. Co-administration of surfactant materials has been shown to affect absorption from solid dosage forms. Various effects of surfactants have been reviewed by Gibaldi and Feldman[23-24]. Surfactants are capable of interfering with the integrity of the mucosal membrane[25-28] as well as interacting with drug formulations. Oils have been shown to alter gastric emptying rate[13,30,31]. Digestion of the oil appears to be significant in connection with the latter phenomenon and with drug absorption from oils[32-33]. Therefore a great variety of factors may influence absorption from emulsions. In a recent review S.S. Davis[34] described the use of emulsions as vehicles for oral administration of drugs and concluded that, in certain circumstances, emulsions have advantages over conventional dosage forms. Emulsions may reduce gastric irritation of certain drugs, simplify problems of taste-masking or problems caused by chemical instability of drug molecules in aqueous media. Gastrointestinal digestion of large molecules such as insulin or heparin may be reduced[35-37]. However emulsions are rarely used for oral preparations in practice due to difficulties in formulating physically stable systems.

Many commonly used herbicides have low solubilities in water[38]. Concentrated aqueous solutions or solubilized systems cannot be prepared with such herbicides[39] and transportation of working solutions in bulk is impractical. Therefore lipophilic herbicides are dissolved in organic

solvents (xylene or trimethylbenzenes) including surfactants to produce 'self-emulsifying' formulations. These 'emulsifiable concentrates' can be dispersed easily in local water prior to crop spraying.

It was considered that a similar pharmaceutical vehicle might be prepared which, when placed in soft gelatin capsule, would subsequently emulsify within the gastric contents. Such a formulation would present a lipophilic drug in oily solution with a large interfacial area across which diffusion could take place. It was proposed that a 'self-emulsifying' vehicle may increase the rate *and/or* extent of absorption of a poorly water soluble drug. The aims of the present study were to attempt to formulate an efficiently emulsifying formulation with pharmaceutically acceptable materials; to develop objective methods of comparing dispersion properties of self-emulsifying formulations; to determine which physical properties affect dispersion properties and to carry out preliminary biological studies designed to assess whether such a formulation has potential practical merits.

1.2. SELECTION OF MATERIALS

It was considered that an ideal 'self-emulsifying' vehicle would have the following characteristics:

1. The vehicle would comprise inexpensive, readily available materials which were well tolerated orally and would not result in toxicity following chronic administration.
2. The mixture would be a clear, monophasic liquid at ambient temperatures in order to avoid physical instability during storage and to facilitate mixing and encapsulation.
3. The vehicle would have good solvent properties which would allow presentation of drug substances in solution.
4. The formulation would be inert with respect to the soft gelatin capsule.

Generally 'self-emulsifying' formulations consist of one or more surfactant(s) dissolved in an oily solvent. There are few surfactants which are considered acceptable for oral ingestion. Non-ionic surfactants are thought to be more appropriate than ionic materials; certain non-ionic materials have been approved for use in food and oral pharmaceutical products[40]. The selection of oils was also restricted. The organic solvents used in herbicidal self-emulsifying formulations are highly toxic and unsuitable for oral ingestion.

Initially a series of commonly used non-ionic materials based on esters of sorbitan were selected as acceptable surfactants. Self-emulsification efficiencies of mixtures of the latter surfactants with acceptable oils (vegetable oils, liquid paraffin) were screened by a subjective method*.

Oxyethylenated surfactants based on oleate esters of sorbitan, comparatively fluid due to the unsaturated oleate chains, appeared to be the most useful surfactants. On the basis of ease of emulsification the triglyceride oils appeared to be more suitable than liquid paraffin. In addition the triglycerides had higher solvent capacity than liquid paraffin. The fluid, medium chain triglyceride oil, Miglyol 812, in combination with polyoxyethylene-20-sorbitan trioleate (Tween 85) was the system of choice.

Previous workers have suggested that self-emulsifying systems are best formulated using a combination of an hydrophilic and a lipophilic surfactant[42]. Sorbitan trioleate (Span 85) was selected as a suitable lipophilic surfactant; the combination Miglyol 812/Tween 85/Span 85 was proposed as a model system. Certain related surfactants and oils were selected for comparison with the above model.

---

* The method used was a modification of that adopted for assessment of pesticide formulations[41]. One millilitre of monophasic oil/surfactant mixture was lowered onto the surface of 99ml distilled water contained in a stoppered, glass 100ml measuring cylinder. Groups of cylinders were rocked gently using an automated device for one minute. Resultant emulsions were then awarded an appropriate score on the basis of their visual appearance.

1.3. SCOPE OF PHYSICAL RESEARCH

The efficiency of emulsification of oil/surfactant mixtures in aqueous media has been characterized by two measurements; the rate of emulsification in a stirred cell and the particle size of the resultant emulsion at equilibrium.

By measuring the emulsification properties of a wide range of oil/surfactant mixtures in distilled water it was possible to assess the importance, to emulsification, of the various factors listed below.

1. Viscosity of the oil/surfactant mixture
2. Density of the oil/surfactant mixture
3. Hydrophile-lipophile balance of the surfactant component
4. Concentration of the surfactant component
5. Identity and nature of the oil component

Initially the effects of mixture composition on emulsification of Miglyol 812/Tween 85/Span 85 mixtures in distilled water at 25°C were examined. The influence of temperature was also studied. Subsequently the surfactant component was varied by substituting a more hydrophilic material, Tween 80, for Tween 85. In turn the importance of the oil component was examined by substituting a series of related materials for Miglyol 812. The oils were selected to give a range of densities, viscosities and

polarities.

Returning to the properties of the model system, the effects of moisture content and drugs dissolved within the vehicle were studied. These experiments provided useful information, indicating the likely mechanism of efficient self-emulsification. Finally the influence of ionic species present in the aqueous phase was examined.

Studies of oil/surfactant/water phase behaviour were undertaken corresponding to the range of emulsification experiments.

1.4. BIOPHARMACEUTICAL CONSIDERATIONS AND SCOPE OF PRESENT STUDY.

Two parameters can be used to describe the efficiency of absorption from the gastro-intestinal tract. These are the rate at which a drug is absorbed and the extent of absorption in relation to the amount initially available for absorption. Neither parameter is directly related to the therapeutic efficacy of a particular medicament but they are indicative of the availability of a drug for distribution throughout the body. A measure of the absorption process can be obtained by determining drug concentration in the circulating plasma, at various times after drug administration. However, rapid metabolism in the liver ('first-pass' effects) may distort results and a major disadvantage is the inconvenience of sampling plasma repetitively. Examination of urinary excretion of drug and/or metabolites has also been used to measure the extent of absorption. However for most drugs elimination (metabolism and excretion) is a slow process in comparison with absorption. In such cases one cannot study absorption rate by examining urine contents.

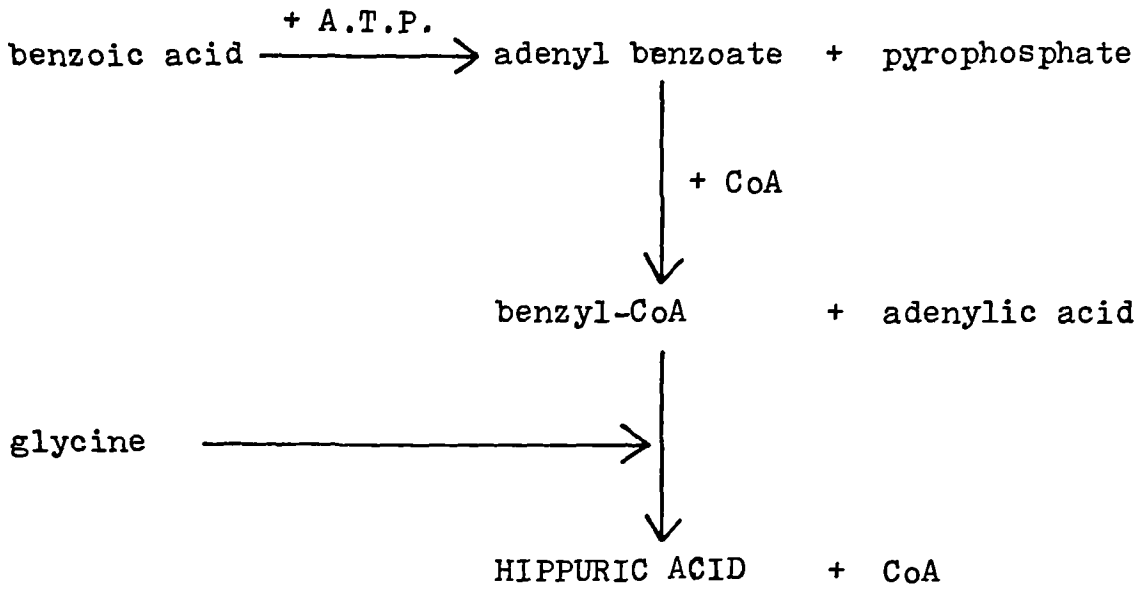
Present biopharmaceutical experiments were restricted to examination of urine contents.

For the purpose of evaluating absorption from self-emulsifying vehicles there seemed to be two salient experiments. The first is to select a drug which is

eliminated rapidly in order to observe any changes in the absorption rate. (It was accepted that such a drug model would probably be comparatively well absorbed from most formulations). Secondly one could select a drug which is known to be poorly absorbed and compare the extent of absorption by determining the total amount of drug and/or metabolites appearing in the urine. A version of the former experiment has been conducted during the current project.

Benzoic acid was selected as a suitable model drug. The metabolism of benzoic acid is simple and well documented. Its elimination is rapid. It has been used previously, in doses commonly up to 5g, as liver and kidney function tests for the above reasons[43-44]. The drug also has appreciably higher affinity for non-polar solvents than it has for water and as such is representative of drug materials which may benefit from formulation in self-emulsifying mixtures.

Benzoic acid is excreted totally in the urine, primarily as the glycine conjugate (hippuric acid)[43-46]. In low doses, of up to 2g, 95-100% of the dose has been recovered as hippuric acid in the urine. The metabolic pathway is represented below.



The abreviations represent adenosine triphosphate and coenzyme A.

Production of glycine seems to be stimulated by the presence of benzoic acid[47] and the natural pool can be saturated after high doses (above 2g) of benzoic acid[46]. Amsel and Levy[46] showed that simultaneous administration of glycine increases the elimination rate after a 5g dose of benzoic acid. However at low doses the glycine pool is not saturated and the glycine conjugation is not a rate limiting process. Wu and Elliott[45] showed that the rate of elimination of hippuric acid after 2g benzoic acid was not significantly different to that after the administration of the equivalent amount of hippuric acid. After larger doses of benzoic acid a greater proportion is excreted as unchanged drug or as benzoyl glucuronide[43-46].

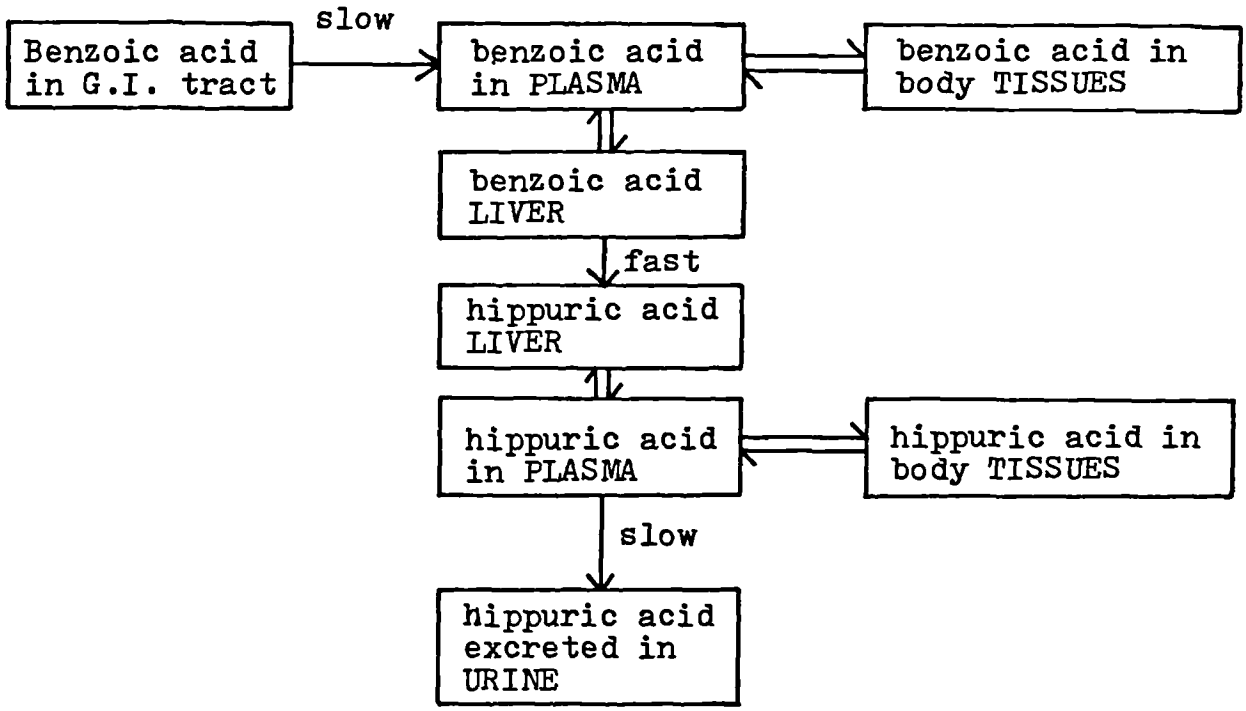
Wu and Elliott determined that the conjugation and subsequent excretion of Benzoic acid as hippuric acid had first order kinetics with rate constants as follows:

rate of hippuric acid synthesis $K_s = 10.5hr^{-1}$

rate of hippuric acid excretion $K_e = 2.7hr^{-1}$

During the work of Amsel and Levy[46] the excretion rate constant was found to be $K_e = 1.4hr^{-1}$ although the figure quoted by Wu and Elliott is based on a substantially greater volume of research.

The dose of benzoic acid used during the current research was 250mg. For such low doses the distribution of drug and its metabolites can be represented by the figure below. The absorption process and the hippuric acid excretion processes being rate limiting factors.



The elimination of benzoic acid following administration in three different formulations has been studied using six healthy, male volunteers. Two additional formulations were studied using three of the volunteers.

CHAPTER TWO

EMULSIFICATION and NON-IONIC SURFACTANTS

2.1. CONVENTIONAL EMULSIFICATION

Emulsification is governed by two competing processes; the disruption of a bulk fluid to form finely dispersed droplets and the coalescence of these droplets to reform the bulk fluid. Emulsions are thermodynamically unstable but may be highly kinetically stable (or metastable) in which case coalescence takes place slowly. The formation of an emulsion can be regarded as three distinct processes: a) the formation of slicks; b) their break up into individual droplets; c) the separation of the droplets to form a homogeneous dispersion[48]. Demulsification is the result of a series of reverse steps: a) creaming, which may or may not occur; b) flocculation and c) coalescence. The theory of emulsion stabilization and kinetics of the coalescence process is well developed, not least because coalescence takes place relatively slowly and can be studied easily. In contrast the theory of emulsion formation is less certain. One reason for this is the variety of equipment used for emulsification. For a cosmetic or pharmaceutical cream (when interfacial tension is not extremely low) the theoretical work required to form an emulsion is approximated by equation 2.1.

$$\Delta G \text{ form} = \gamma.\Delta A \equiv \gamma.4\pi r^2.N \quad \ldots.(2.1.)$$

where γ is the interfacial tension between the two phases, A the interfacial area and N the number of particles of mean radius r.

In practice the work required to form the emulsion may be 1,000 times greater than ΔG form due to work spent mixing the fluids and lost in the form of heat. Consequently the course of the emulsification process depends heavily on the particular method employed and the conditions used. In order to promote emulsification in practice conditions of high shear are employed and homogenisers typically include baffles to encourage turbulent flow.

Studies concerned with emulsification have fallen broadly into two categories. Firstly an empirical approach to practical emulsification aiming to optimise the efficiency of mixing equipment. Secondly the study of the deformation and rupture of bulk fluids in conditions of specialised, simplified flow. A brief description of the conclusions of this work follows; Becher[49] and Gopal[50] have reviewed research in more detail.

2.2. MODEL STUDIES OF EMULSIFICATION

Ultrasonic emulsification has been explained by disruption of interfaces due to compression and stretching of liquids and seems to be linked with cavitation within the continuous phase[49-51]. The effect of ultrasonic frequency on emulsification is complex; certain frequencies promote demulsification. However if a single frequency and intensity is used the effect is consistent. For this reason ultrasonic methods have been used to verify calculations of emulsion growth. Some authors have represented the emulsification process by equation 2.2.

$$\frac{\delta N_t}{\delta t} = B.N_t - A.N_t^2 \qquad \text{.....(2.2.)}$$

This equation includes terms relating dispersion and coalescence[52-53]; N_t is the number of particles at time t and A and B are rate constants. The equation describes a process which reaches a limiting particle number (and therefore particle size) which has been shown to occur widely in practice. Data for ultrasonic emulsification fitted the model and this has inspired a recent study by Tsukiyama and co-workers on emulsification using a Rushton-type impellar mixer[54]. Other authors have considered that Equation 2.2 is too simplistic to represent emulsification by a process of high mechanical shear[48].

Hydrodynamic interfacial instability can be classified into four main types[50] which are fundamental to emulsification. Each type has characteristic features brought about by certain conditions and has been studied in detail. In conditions of turbulent flow, common to emulsification processes, all types of hydrodynamic instability are operative. In conditions of specialised flow one type of instability may be prevalent although the classes of instability are inter-related.

Liquid jets are generally unstable due to varicose deformations of the interface which cause sinusoidal perturbations of the radius of a cyclindrincal jet. The result can be break up of the jet into a line of droplets[55](figure 2.1.).

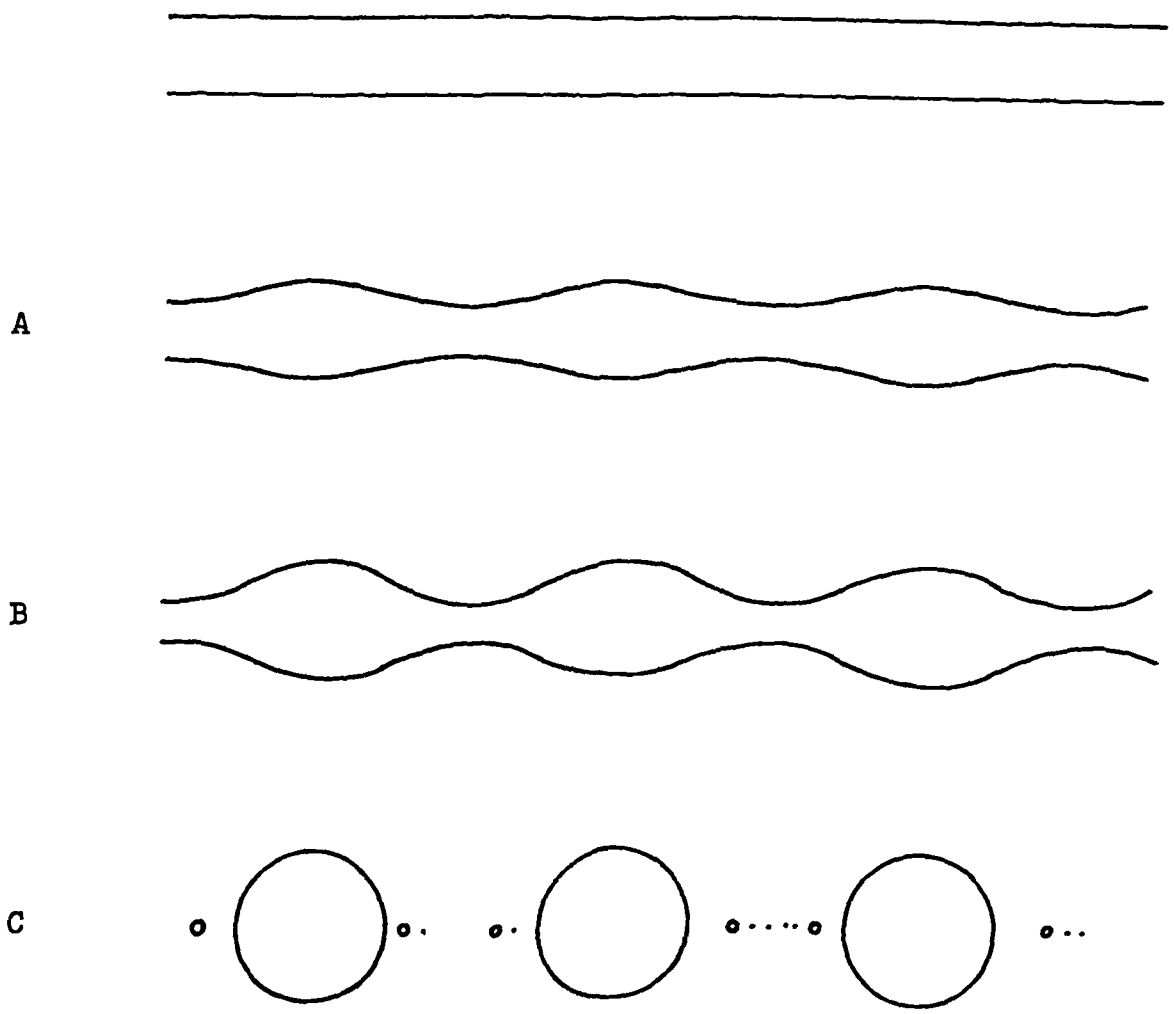
When one liquid is injected into another immiscible liquid from a narrow orifice the relative velocity of flow is extremely important. Break up of the jet is controlled by inertial and viscous forces. A critical velocity has been described by Richardson[56] above which fine droplets result. An empirical relationship estimates this velocity V_o:

$$\frac{\eta_d}{(\rho_d.\gamma.D)^{\frac{1}{2}}} = 2000\left[\frac{\eta_d}{V_o.\ \rho_d.D}\right]^{\frac{4}{3}} \quad .....(2.3)$$

Where D is the nozzle diameter, ρ_d and η_d are the density and viscosity of the dispersed phase and γ is the interfacial tension. Break up of liquid threads has

Figure 2.1. The break-up of liquid jets.

Varicose deformations (A) are amplified (B) and result in droplet formation (C).



been examined as a function of mean size of resultant particles[57-58]. Generally particle size decreased with increasing velocity of jet flow, decrease in interfacial tension, increase in the density difference ($\rho_c - \rho_d$), decrease in continuous phase viscosity and decrease in nozzle diameter.

Rumscheitd and Mason have examined deformation and rupture of droplets in laminar flows[59]. A droplet bursts when the pressure drop across the interface due to shearing is greater than the interfacial tension. The viscosities of the dispersed and continuous phases are of great importance to the mode of droplet deformation as demonstrated by photographs[59]. In conditions of plane flow droplets deform to prolate or oblate spheroids[60] although as soon as flow becomes turbulent the deformations become irregular. These properties are likely to be important to the later stages of emulsification whereas the break-up of threads may be more relevant to the initial stages of emulsification.

Practical emulsification occurs in turbulent conditions although studies using simple mixers have shown that the effects of physical properties in practice are similar to those described in fundamental studies. Roger, Trice and Rushton[61] have examined the effect of agitation on interfacial area for a wide variety of emulsions formed by an impeller in various cylindrical tanks. Data was correlated empirically. Increase in the density difference

$(\rho_c - \rho_d)$ caused an exponential increase in interfacial area and an attempt was made to correlate the effect of agitation in terms of the Weber number : $D_i^3 . V_i^2 . \rho_c/\gamma$ where D_i and V_i are the impeller diameter and speed respectively and γ is the interfacial tension. Several other studies using mixers or homogenisers have yielded similar results[62-65]. The net effect of viscosities of the continuous (η_c) and dispersed (η_d) phases is complex. Calculations suggest that increase in η_d/η_c should retard emulsification. In fact this does not always occur. Increase in η_d may hinder coalescence in some cases compensating for less efficient break-up of droplets.

The majority of the hydrodynamic instability studies mentioned above have been carried out on simple mixtures of two immiscible liquids (some of the impeller or homogenisation experiments involved surfactants in small quantities). Whilst these studies have provided useful information with regard to emulsification, in some cases appropriate use of surfactants to lower interfacial tension may overwhelm the effects of the other physical properties described above. Surfactants are strongly adsorbed at oil/water interfaces and lower the interfacial tension as given by the Gibbs adsorption isotherm:

$$-\delta\gamma = \Gamma . RT . \delta \log_e a \quad \ldots\ldots\ldots (2.4.)$$

where Γ is the surface excess (amount adsorbed per unit area), a is the activity of the solute in the bulk phase, R and T are the Gas constant and temperature (^oK). The case of extremely low interfacial tension is discussed in the next section.

2.3. SPONTANEOUS EMULSIFICATION

Reports of spontaneous emulsification have appeared in the literature, sporadically, since the late 19th century. Reading through earlier work one is conscious of the wide range of mixtures of materials which are grouped together under the heading, 'spontaneously emulsifying'. Explanation of these phenomena has not been easy, largely because the dominating mechanisms depend on the particular materials described in each case.

The earliest reports of spontaneous emulsification[66] were explained by Quincke[67], who suggested that the interfacial instability, manifested by kicking and streaming of emulsion droplets from the interface, was due to uneven adsorption of solutes at the interface. Local reductions in interfacial tension caused violent spreading and interfacial turbulence resulting in emulsification. This effect may explain some types of spontaneous emulsification; that which occurs when materials are impure or as in the case of Gad's work[66], when soap is formed in situ at the oil/water interface. More recently Sternling and Scriven[68] and other authors[69] have discussed the role of interfacial turbulence mathematically in efforts to explain the effects of adsorbed solutes on the hydrodynamic stability of interfaces.

Interfacial turbulence however is not essential during spontaneous emulsification[70-73]. The study of McBain and

Woo[72] illustrates this point. Emulsification took place gradually when mixtures of methanol and toluene were layered gently on to water. The droplets appeared in either the water or oil phase depending on the concentration of methanol dissolved in the toluene No turbulence was observed during the emulsification. Such behaviour is explained by molecular diffusion of one or more components across the interface and is typical of mixtures which are 'partially miscible'[74]. In the case of the toluene/water/methanol mixtures, methanol acted as a co-solvent for toluene and water. Molecular diffusion of toluene and methanol occurred across the interface and, after the mixture became saturated with water, toluene was liberated as emulsion droplets. This is a common (but specialised) form of spontaneous emulsification.

Other examples of spontaneous emulsification were not so easily explained[75-79] although in most cases low interfacial tension seemed an important prerequisite and (by refering to equation 2.1) Stackelburg[80] proposed that "negative interfacial tension" might explain his observations.

J.T. Davies and co-workers[81-82] attempted to collate the available observations and suggested that there were three main mechanisms by which spontaneous emulsification could take place, although these were not mutually

exclusive; interfacial turbulence, diffusion and stranding and negative interfacial tension. Other authors[83-85] were sceptical about the concept of negative interfacial tension and indeed it is now widely accepted that it is unnecessary to postulate negative interfacial tension, although some authors continue to do so[86-88].

Certainly a low value of interfacial tension is necessary for spontaneous emulsification whether facilitated by adsorption of surfactant at the interface or by mass transfer (which is of continuing importance[89-91]). But postulation of negative interfacial tension is to simplify the forces involved in emulsification[92]. The dispersion of droplets represents an entropy gain due to increased translational degrees of freedom. Thus the free energy of emulsion formation is more correctly given by:

$$\Delta G \text{ form.} = \gamma . \Delta A - T . \Delta S \text{disp.} \quad \ldots\ldots\ldots (2.5)$$

where ΔA is the change in interfacial area resulting from the formation of the emulsion and ΔSdisp. is the entropy gain in the system resulting from the dispersion of the droplets.

For large droplets when there is a large density difference between the continuous and dispersed phases work will also also be required to disperse the droplets; to overcome the external gravitational forces (ΔHdisp). In this case:

$$\Delta G_{form.} = \gamma . \Delta A + \Delta H_{disp.} - T . \Delta S_{disp.} \quad .....(2.6)$$

It would seem that there is a critical value of γ, (γcrit.), below which true spontaneous emulsification takes place[48]; below γcrit the value of $\Delta G_{form.}$ is negative. Therefore a negative value of γ is not necessary for spontaneous emulsification. (Rosano has noted that if γ were ≤ 0 then one would not expect formation of spherical emulsion droplets[93]). Reiss[94] has presented theoretical calculations which arrive at an equation describing the value of $- T.\Delta S_{disp}$ allowing prediction of values of γcrit:

$$\Delta G_{form.} = \gamma . \Delta A - T . \Delta S_{disp} = \gamma . \Delta A - kT . N . K'$$

where,

$$K' = \log_e \left\{ \frac{V . n^{1/2}}{\nu} . \left(\frac{12}{\pi}\right)^{3/2} . (1 - \phi) . \exp\left[\frac{3\phi(\phi-2)}{2\phi(1-\phi)^2}\right] \right\} - \log_e N + 1$$

Where N is the number of particles of dispersed phase each containing n molecules, V is the total volume of the two liquid phases, ν is the molecular volume of the dispersed phase and ϕ is the volume fraction of the dispersed phase.

Using Reiss' calculations the value of γcrit for a typical oil/water/surfactant system is below 1 mNm^{-1} and probably of the order 10^{-4}-10^{-2} mNm^{-1}. This would explain the difficulty past authors have experienced when trying to determine values of interfacial tension for

spontaneously emulsifying mixtures; all available techniques are insensitive to such small values.

One important implication of Reiss' theory is that spontaneous emulsification is a dynamic process. Therefore the value of γ may be transiently lower than γcrit but may become larger than γcrit at a certain point during the process. Perhaps co-surfactant may cease to cross the interface, or adsorbed surfactant could become depleted. At either point if $\gamma > \gamma$crit then emulsification would cease. Thus the theory does not imply that emulsification should continue until all of the dispersed phase is emulsified as fine droplets[95].

One can imagine that when γ is slightly higher than γcrit, emulsification would take place easily although not truly spontaneously. 'Self-emulsifying' formulations need not be spontaneously emulsifying, as mentioned in chapter 1.

For conventional oil/water/surfactant mixtures (in the absence of a co-solvent) the lowering of γ to reach γcrit often depends on adsorption of the surfactant at the oil/water interface. In recent years spontaneous emulsification has been linked with the formation of mesomorphic phases at the interface[42, 96-98]. Such interfacial phenomena may be an important way of reducing γ to low values for certain materials, although at present these suggestions are speculative.

2.4. SOME PROPERTIES OF NON-IONIC SURFACTANTS

Molecules which contain hydrocarbon chains and also aliphatic hydroxyl groups may be polyoxyethylenated to give a wide variety of amphiphilic materials collectively known as non-ionic surfactants.

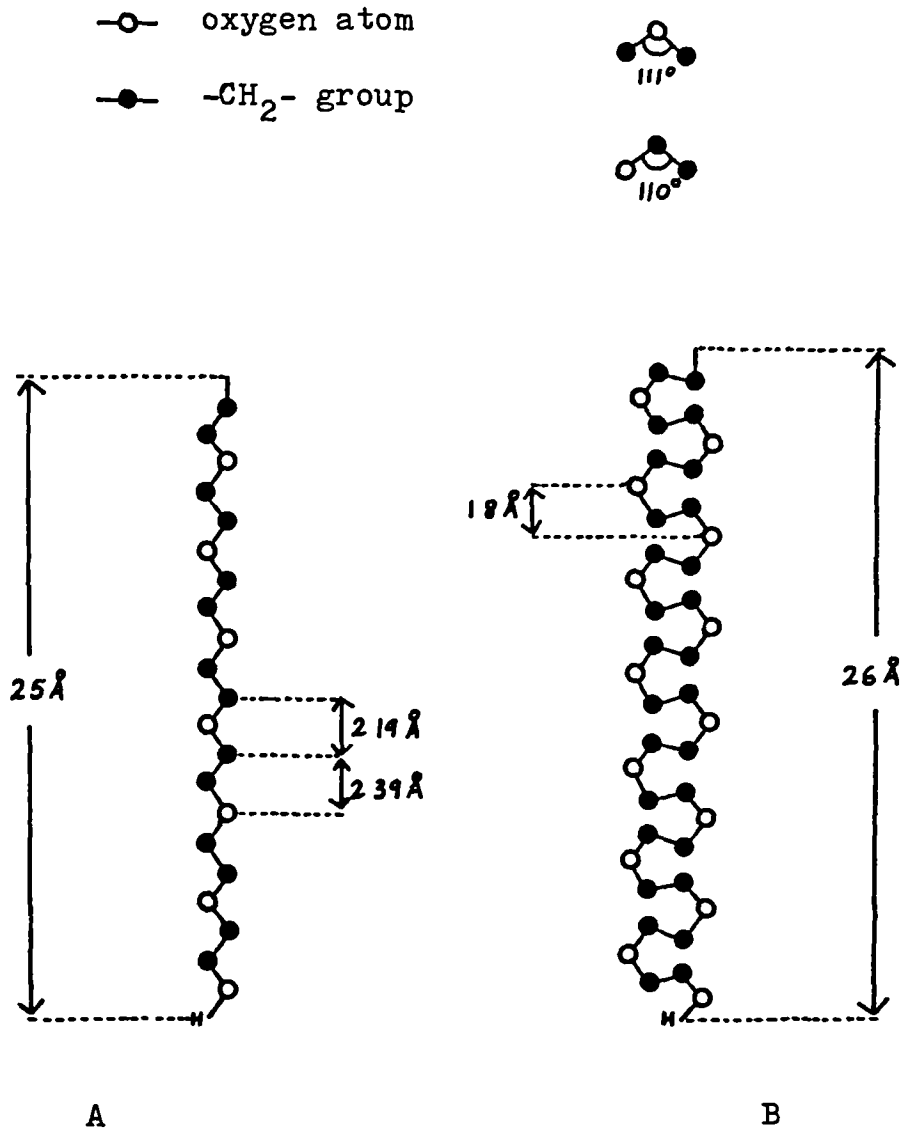
$$R'\text{——}X\text{——}OH \longrightarrow R'\text{——}X\text{——}(OCH_2CH_2)_n - OH$$

Whilst their molecular structures may vary considerably their general properties are similar, due to the fact that their lipophilic and hydrophilic moieties are, essentially, the same. The amphiphilic character of each material is governed by respective affinities for oil and water. An arbitrary gauge, the hydrophile-lipophile balance (HLB), was conceived by Griffin[99-100] and is widely used as a guide to amphiphilic character[101].

Oxyethylenation is a polymerization process which therefore results in a mixture of molecules of varying oxyethylene content. Industrial surfactants are just such mixtures. Certain authors have produced comparatively pure materials for research purposes by molecular distillation or solvent extraction[102]. Generally industrial surfactants have similar properties to materials of narrow molecular weight ranges. However boundaries between phase changes are less sharp in mixtures containing industrial surfactants[103].

The hydrophilic moieties of many non-ionic surfactants are provided by oxyethylene chains which have a high affinity for water. The dipoles of the ether bridges within the oxyethylene chains are able to form hydrogen bonds with the dipoles of water molecules[104-106] although this does not fully explain the miscibility of water and polyoxyethylene. The closely related polymers polyoxymethylene and polyoxypropylene are poorly soluble in water[107]. One might expect that polyoxymethylene would have greater hydrophilic character than polyoxyethylene. The reason for this anomaly is not clear, although the structuring of water may be important. Polyoxyethylene chains in aqueous solution exist in a helical conformation with seven (CH_2CH_2O)- groups per helix revolution[108]. In this conformation the distance between oxygen atoms (0.277nm) is exactly the same as the oxygen-oxygen distance in the tetrahedrically bonded wurtzite structure of water. Therefore there may be a relationship between the long range order of water structure and solubility of polyoxyethylene[109]. In solution chains usually conform to a compressed 'meander' configuration when above nine units in length. An extended 'zig-zag' configuration is formed by short chains[110-111] (figure 2.2.). The helical form of longer 'meander' chains is important because water can become trapped within the helix. Therfore there are two pools of water associated with polyoxyethylene; that hydrogen bonded to ether oxygens and that trapped within helical chains[106].

Figure 2.2. Zig-Zag (A) and meander (B) configurations of polyoxyethylene



The affinity of polyoxyethylene chains for water is sensitive to temperature[108]. As temperature increases there is a steady decrease in the hydrogen bonding between ether oxygens and water. This phenomenon has profound effects on the properties of non-ionic surfactants. A typical example of the phase behaviour when a non-ionic surfactant ($C_9H_{19}.C_6H_4O.(CH_2CH_2O)_{9.2}.H$) is mixed with water is shown in Figure 2.3. (redrawn from ref.112). Less hydrophilic surfactants may form more extensive areas of liquid crystalline phase which modify the appearance of the phase diagram although the main features are similar. (For example, Figure 2.4. shows the phase diagram for $C_{10}H_{21}.O(CH_2CH_2O)_3.H$ and water[113]).

Phase region, I_w, in Figure 2.3. represents a micellar solution of surfactant in water. If temperature is increased the phase will become turbid due to increased micelle size[114]. At a characteristic temperature for the particular composition, known as the cloud point, phase separation of the surfactant occurs (region II_{w+s}); micellization is no longer thermodynamically favoured[115-116]. Figure 2.4. shows that the cloud point is lower for the less hydrophilic surfactant and this follows a general trend[117]. Phase behaviour of surfactants with different HLB values is similar, but corresponding phase changes occur at higher temperatures with increasing HLB. If a surfactant is sufficiently lipophilic it will not form aqueous micelles at all and is immiscible with water at all temperatures (i.e. its cloud point is below

Figure 2.3. Phase diagram of water and the surfactant $C_9H_{19}.C_6H_4.O.(CH_2CH_2O)_{9.2}H$ (redrawn after Shinoda[112])

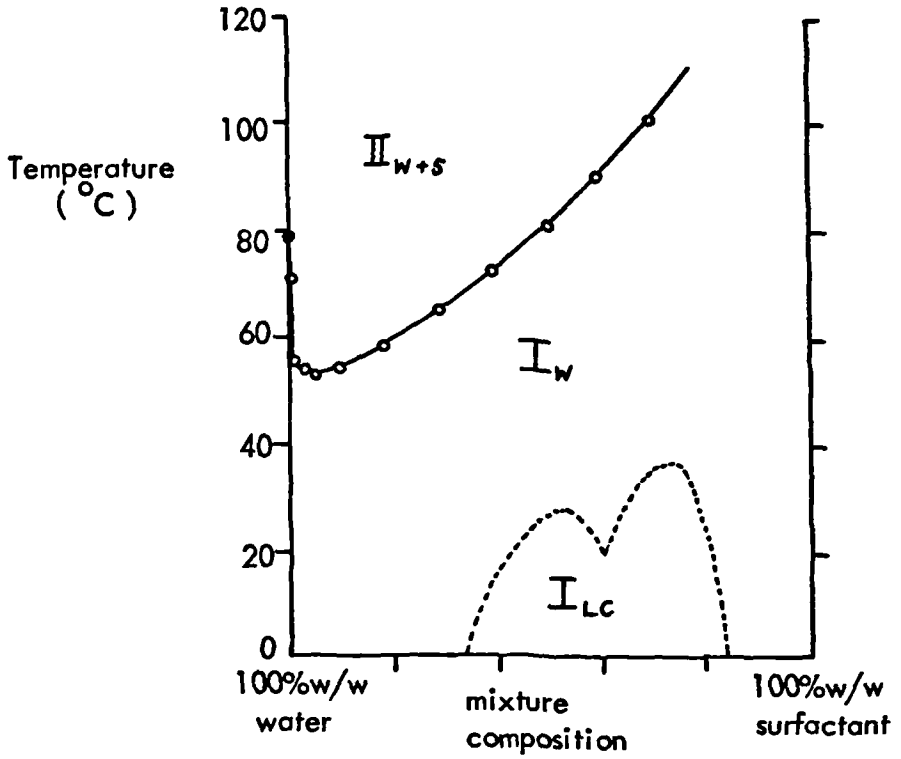
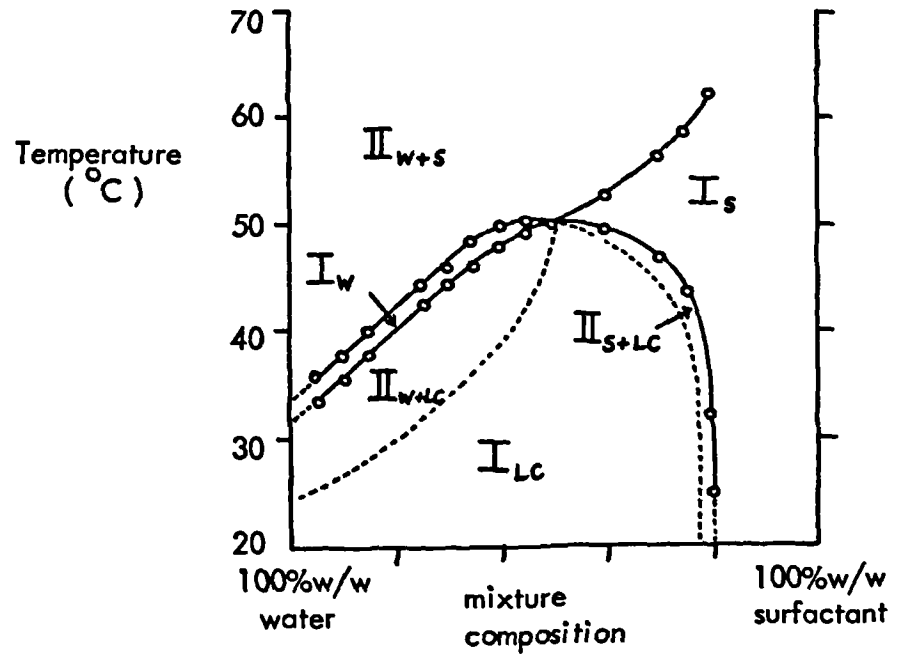


Figure 2.4. Phase diagram of water and the surfactant $C_{10}H_{21}.O.(CH_2CH_2O)_3.H$ (redrawn after Ali and Mulley[113])

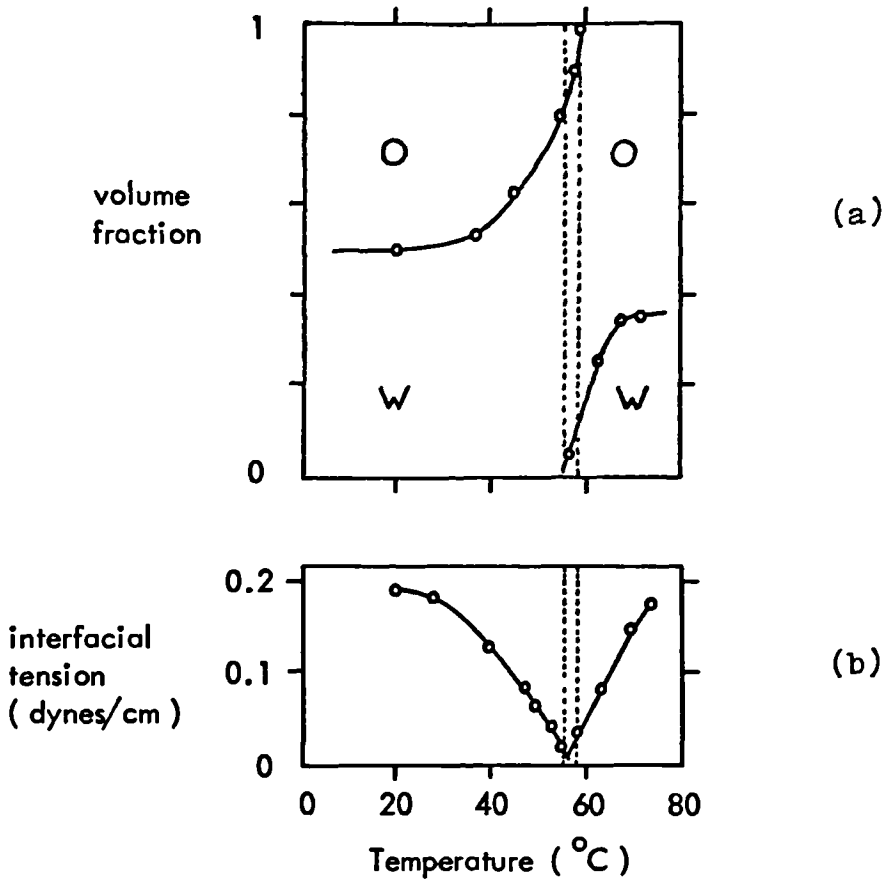


the freezing point of water).

When a third component, an oil, is introduced into a non-ionic surfactant and water mixture the phase behaviour, whilst more complicated, conforms to a general pattern which has been revealed largely by Shinoda's school[117-123]. Shinoda's concepts have become accepted and widely used by other workers interested in emulsions or 'microemulsions'[95, 124-128]. The phase behaviour of oil/water/non-ionic surfactant mixtures can now be explained and in many cases predicted although as Sherman and co-workers have pointed out, in some cases interfacial association in mixed surfactant systems may complicate behaviour[129-130]. Phase behaviour of oil/water/surfactant mixtures depends heavily on the nature of the oil as well as the surfactant HLB value, temperature and mixture composition.

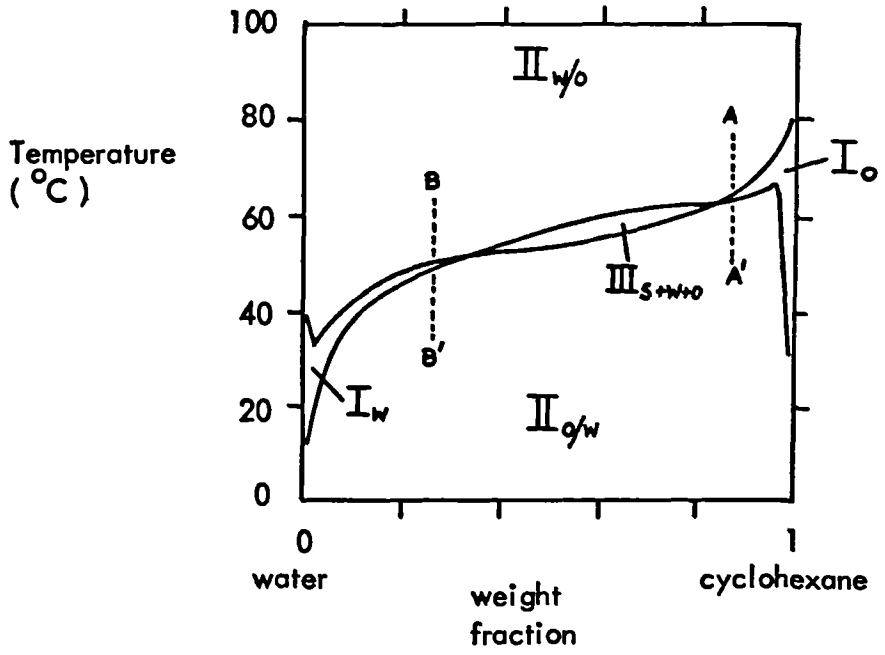
The effect of temperature on a mixture composed of 47.5% cyclohexane, 47.5%^{w}/w water and 5%^{w}/w polyoxyethylene -8.6- nonylphenylether is shown in Fig. 2.5. (redrawn from ref.115). At temperatures of approximately 20^{o}C the mixtures consist of two phases with roughly equal volumes; an oil phase and an aqueous phase containing the majority of the surfactant in micellar solution. When homogenized the mixture forms a 'meta-stable' oil-in-water emulsion. If the temperature is increased the amount of oil solubilized within the aqueous micelles increases and the

Figure 2.5. The effect of temperature on the volume fractions (a) of water (W) and oil (O) and the interfacial tension between these phase (b)



(5%^{W}/w polyoxyethylene (8.6) nonylphenylether, 47.5%^{W}/w water and 47.5%^{W}/w cyclohexane)

Figure 2.6. The phase diagram of water and cyclohexane in the presence of 5%^{W}/w polyoxyethylene (8.6)- nonylphenylether as a function of temperature



water phase appears to grow at the expense of the oil phase. A sharp change in phase volume ratio takes place above 40°C accompanied by a fall in interfacial tension. At 55°C (which is close to the cloud point of the surfactant in water) three phases exist. These are: a) the remnants of the oil phase; b) a phase consisting of surfactant with large amounts of solubilized oil and water, occupying about 80% of the volume of the mixture (which Shinoda calls the 'surfactant phase') and c) an aqueous phase containing a small amount of surfactant (<0.1%). 55°C represents a phase-separation temperature for surfactant in water. at 58°C only phases b) and c) remain (that is, all the oil has been incorporated into the 'surfactant phase'). Between 55° and 58°C the interface between phases is poorly defined, pseudo-continuous and therfore the value of interfacial tension is very low. Emulsification is favoured in this region although emulsions once formed are not as stable as those produced at lower or higher temperatures[124-133] because anchoring of surfactant at the oil/water interface is weaker[126]. Above 58°C the 'surfactant phase' becomes progressively 'oily' as water is squeezed out. Phase c) thus grows in volume and there is an accompanying rise in interfacial tension. When the temperature is as high as 70°C the water phase volume becomes quite large and the other phase consists of oil containing most of the surfactant as 'inverse micelles'. The mixture forms a water-in-oil emulsion above 60°C. In summary, the rise of temperature between 40° and 70°C results in a transfer

of surfactant from the water to the oil phase. The region 55-58°C is a Phase Inversion Region (the approximate temperature is known as the Phase Inversion Temperature - PIT).

A more complete phase diagram for the same materials is shown in Figure 2.6. (redrawn from ref.131). The weight of surfactant is kept constant but the effect of the oil/water weight ratio is described. At low proportions of water and oil the three-phase region (III) gives way to isotropic phases (I_o and I_w phases respectively). These phases are thermodynamically stable micellar solutions with solubilized water and oil in either case. Thus if conditions change from B' to B or A' to A by increase in temperature then true phase inversion takes place. This occurs at different temperatures illustrating the importance of mixture composition on PIT. The behaviour of cyclohexame/water/polyoxyethylene -8.6- nonylphenylether mixtures is typical of other oil/water/non-ionic surfactant mixtures. The PIT depends on the natures of the particular materials. Increase in hydrophilic character of the surfactant raises the PIT. Indeed a diagram analogous to Figure 2.6 can be constructed by plotting the number of oxyethylene units for a homologous series of surfactants against oil/water weight fraction at constant temperature[131].

The phenomenon of phase inversion has been rationalised by envisaging a divided total interfacial tension[109,134-137];

an interfacial tension between the oil and the non-polar part of the surfactant (γ O/N) and another between the water and the polar part of the surfactant (γ W/P). At low temperatures at a plane interface,

$$\gamma \text{ W/P} < \gamma \text{ O/N}$$

thus the interfacial film tends to curve to form oil droplets. At high temperatures the reverse is true favouring formation of water droplets. At the PIT

$$\gamma \text{ W/P} \cong \gamma \text{ O/N}$$

which favours a plane interface. Shinoda proposed that the 'surfactant phase' may be lamellar in form[123,131]. Certainly some birefringence has been observed in the PIT region. However other authors have failed to detect regular layer structures with low angle diffraction techniques. Consequently the structure of the surfactant phase is uncertain. Friberg has suggested that a non-regular bicontinuous structure may explain observations[127]. The nature of such mixtures is very much a matter currently being debated in the literature, particularly in relation to the structure of so-called 'microemulsions'. The effects of temperature on oil/water/non-ionic surfactant systems are closely related to the effects of adding a 'co-solvent' (usually a medium chain alcohol) at constant temperature. Such four component mixtures are currently of considerable interest[138-144] and along with studies on the nature of the 'surfactant phase' should yield useful information regarding spontaneous emulsification.

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1 Water

Water was freshly distilled from an all glass apparatus and held in a glass aspirator reserved for the purpose.

3.1.2 Lipophilic materials

A medium chain triglyceride oil (Miglyol 812) was obtained from Dynamit Nobel (UK) Ltd. This material is manufactured from coconut oil by distilling a fraction of saturated fatty acid mixture and subsequently esterifying the mixture with glycerin[145]. A similar material (Miglyol 840) produced by esterifaction of saturated fatty acids with propylene glycol[146] was obtained from the same source. Both oils were clear liquids at ambient temperatures containing little moisture ($<0.1\%^{w}/w$) or free fatty acids ($<0.3\%^{w}/w$). The composition of the oils as $\%^{w}/w$ esterified fatty acids is shown below[146]. Physical properties of the oils are also tabulated.

FATTY ACID	% $^{w}/w$ composition in oil	
	MIGLYOL 812	MIGLYOL 840
caproic acid (C6)	2% max.	2% max.
caprylic acid (C8)	50-65%	65-80%
capric acid (C10)	30-45%	15-30%
lauric acid (C12)	3% max.	3% max.

PHYSICAL PROPERTY	MIGLYOL 812	MIGLYOL 840
interfacial tension (water/oil) at 20°C	0.0203 Nm^{-1}	0.0219 Nm^{-1}
density at 25°C ± 0.2°C	0.9419	0.9174
viscosity at 25°C ± 0.5°C	17.9 mPa.s	7.4 mPa.s

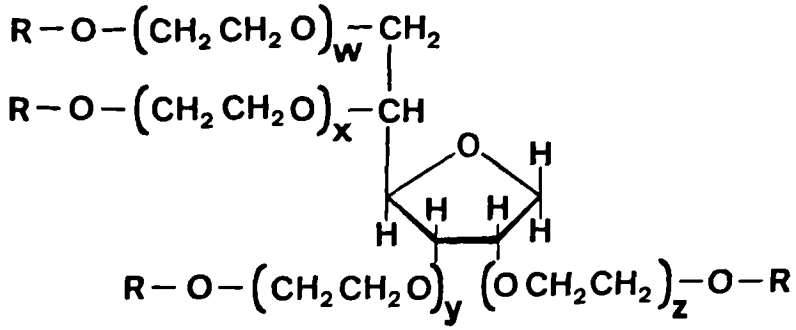
A series of saturated fatty acids of carbon chain length C_6-C_{12} was obtained from Flourochem Ltd. Lipophilic character was more pronounced as carbon chain length increased as indicated by solubilities in water[147]. The acids became more viscous and tended towards waxy solids with increasing carbon chain length as shown by melting point data[148].

FATTY ACID		Melting Point (°C)	Solubility in H_2O (20°C)
Caproic (hexanoic)	C6	0	1.082%$^{w}/_{w}$
Oenanthic (heptanoic)	C7	- 9 to -6	0.242%
Caprylic (octanoic)	C8	16-17	0.068%
Pelargonic (nonanoic)	C9	7-10	0.034%
Capric (decanoic)	C10	30.5-31.5	0.015%
Undecanoic	C11	25-28	-
Lauric	C12	42-45	-

Octane and Octanol were standard laboratory reagent grade supplied by B.D.H. Ltd. Technical grade oleic acid and glyceryl trioleate were also obtained from B.D.H. Ltd. All materials were used as received.

3.1.3 Amphiphilic materials

Food grades of polyoxye thylene-(20)-sorbitan trioleate (Tween 85), polyoxyethylene-(20)-sorbitan monooleate (Tween 80), polyoxyethylene-(5)-sorbitan monooleate (Tween 81) and sorbitan trioleate (Span 85) were used as supplied by Honeywill-Atlas Ltd. These are non-ionic surfactants belonging to a class known as Polyol surfactants[149]. Such materials are synthesised from a nucleus with several hydroxyl groups; six in the case of the sorbitol nucleus. Monoesters of sorbitan are formed by an alcoholysis reaction with triglycerides[150]. Further esterification of the remaining hydroxyl groups is possible as in the case of Span 85 (oleate triesters of sorbitan). A weak hydrophilic moiety is provided by residual hydroxyl groups. Consequently sorbitan fatty acid esters are surface active but predominantly lipophilic. Hydrophilic character of sorbitan esters can be increased by introducing polyoxyethylene chains at hydroxyl sites as in Tween products. The chemical structure of such molecules is indefinite. Esterification and oxyethylenation may occur at any of four hydroxyl sites. A general formula for Tween and Span products is represented below; the constituent groups for an average molecule of each surfactant are tabulated.



	ACID GROUPS - R		No. of polyoxyethylene groups per molecule (w + x + y + z)
	oleic $C_{17}H_{33}CO-$	H-	
Span 85	3	1	-
Tween 85	3	1	20
Tween 80	1	3	20
Tween 81	1	3	5

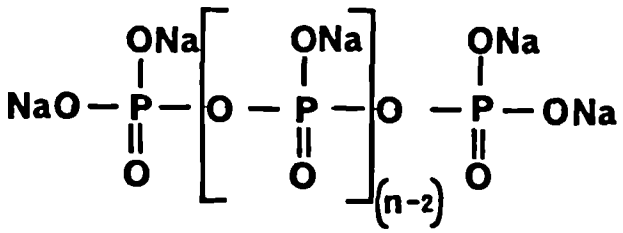
The above amphiphilic materials were clear, yellow-brown liquids at ambient temperatures and did not show obvious signs of deterioration when stored in air-tight jars. Fundamental physical properties of the samples used are tabulated below.

Physical Property	AMPHIPHILIC MATERIAL			
	Span 85	Tween 85	Tween 80	Tween 81
Theoretical mean molecular weight	990	1871	1310	649
H.L.B. value	1.8	11.0	15.0	10.0
Density at 25°C ± 0.2°C	0.9482	1.0242	1.0784	1.0335
Vixcosity at 25°C ± 0.5°C	260mPa.S	262mPa.S	384mPa.S	386mPa.S
Moisture content % w/w	0.3	5.0	3.3	2.8

3.1.4 Other materials

Hydrochloric acid, sodium chloride, calcium chloride dihydrate and sodium hydroxide were SLR grade materials obtained from B.D.H. Ltd.

Glassy sodium phosphate (Calgon S, food grade) having the general structure[151],



where n is approximately 12, was supplied by Albright and Wilson Ltd.

p-hydroxy benzoic acid was supplied by Sigma Ltd. Hippuric acid, benzoic acid and other substituted benzoates were SLR grade materials obtained from B.D.H. Ltd.

Chromatographic solvents, methanol and glacial acetic acid were also obtained from B.D.H. Ltd.

3.2. PHYSICAL METHODS

3.2.1 Equilibrium phase behaviour

Equilibrium phase diagrams as described by Swarbrick[152] were constructed by methods similar to those used by Rosavear and others[153-154]. Constituents were weighed into 15ml glass tubes fitted with PTFE-lined screw-closures. Mixtures were homogenised by heating in a dry oven to approximately 70°C and use of a vortex mixer (Whirlimix, Fisons Ltd.). Mixtures were intermittantly re-mixed whilst cooling to appropriate temperature in a water bath and then left to equilibrate overnight. Phases present were identified by centrifugation and visual inspection or optical microscopy when necessary. Polarising microscopy was used to identify mesomorphic phases.

3.2.2 Preparation of emulsifiable mixtures

Oil-surfactant mixtures were freshly prepared by weight and held in glass tubes fitted with PTFE-lined screw-closures. Temperature was controlled by the use of a water-bath.

3.2.3 Viscosities

Rheological behaviour of isotropic mixtures was examined as a function of shear rate using a Ferranti-Shirley cone-and-plate viscometer. Viscosities of Newtonian materials were determined by measurement of the gradient

of shear stress against rate of shear.

3.2.4 Densities

The densities of isotropic mixtures were determined using an Anton Paar DMA45 densitometer.

Briefly, this instrument contains a water-jacketed, oscillating glass tube. Introduction of gas or liquid into the tube alters the natural frequency of oscillation due to the gross mass change of the tube[155]. The density of a material contained within the tube is calculated from the resulting frequency change. The instrument may be calibrated at a particular temperature using air and water as standard materials.

3.2.5 Physical properties of self-emulsified systems

Introduction

The term self-emulsified has been used to describe emulsions prepared under conditions of slight agitation[32,84,98]. It is likely that some oil/surfactant systems described in the present study emulsify spontaneously in water, although the rate of such a process may be extremely slow. The occurence of true 'spontaneous emulsification' is difficult to establish; small amounts of energy are invariably introduced as separate phases are brought into contact[95]. However the phenomemon of spontaneous emulsification, as defined as a process requiring no external energy, was not of direct importance to the

current study. It was considered appropriate to examine the behaviour of systems in the presence of slight agitation.

An apparatus was constructed to assess the rate of formation of emulsions under controlled conditions. The quality of resultant emulsions was examined using microscopy and a Coulter Nanosizer when appropriate. Experiments were designed such that examinations of emulsion quality could be performed without resorting to dilution of emulsions. Dilution of emulsions has been common practice for sizing purposes but is likely to produce artificial results due, particularly, to migration of surfactant molecules.

3.2.5.1 Comparative assessment of emulsification rate

The apparatus used to assess emulsification rate is described fully in Appendix I. In short, formulations of oil and surfactant were mixed with an aqueous continuous phase contained within a glass cell. Gentle agitation was provided by a rotating glass paddle. During emulsification visible light scattering of the bulk fluid was continuously monitored by plotting intensity of scattered light against time on a chart recorder. The optimum conditions of agitation for the range of experiments were chosen by considering the following criteria:

1. Sufficient agitation was required to mix the bulk fluid homogenously during the emulsification process.

2. A low degree of agitation was desirable for sensitive differentiation between emulsification rates and to avoid turbulence as far as possible.

Generally plots of intensity of scattered light against time were similar to profiles of the dissolution of solids in aqueous media. Characteristically they consisted of a lag phase, a pseudo-linear phase and finally a gradual 'tailing' which reached an asymptote as the emulsion system approached equilibrium. The emulsification processes were compared by means of parameters indicated on a general curve represented by Figure 3.1. These are refered to as:

a) the relative intensity of scattered light at equilibrium, Rel I_{100}

b) emulsification times, $t_{I\%}$ (eg. $t_{0\%}$, $t_{50\%}$, $t_{75\%}$, $t_{90\%}$)

c) emulsification rate of the linear portion $D_{0-50}(sec.\%^{-1}) = (t_{50\%} - t_{0\%})/50$

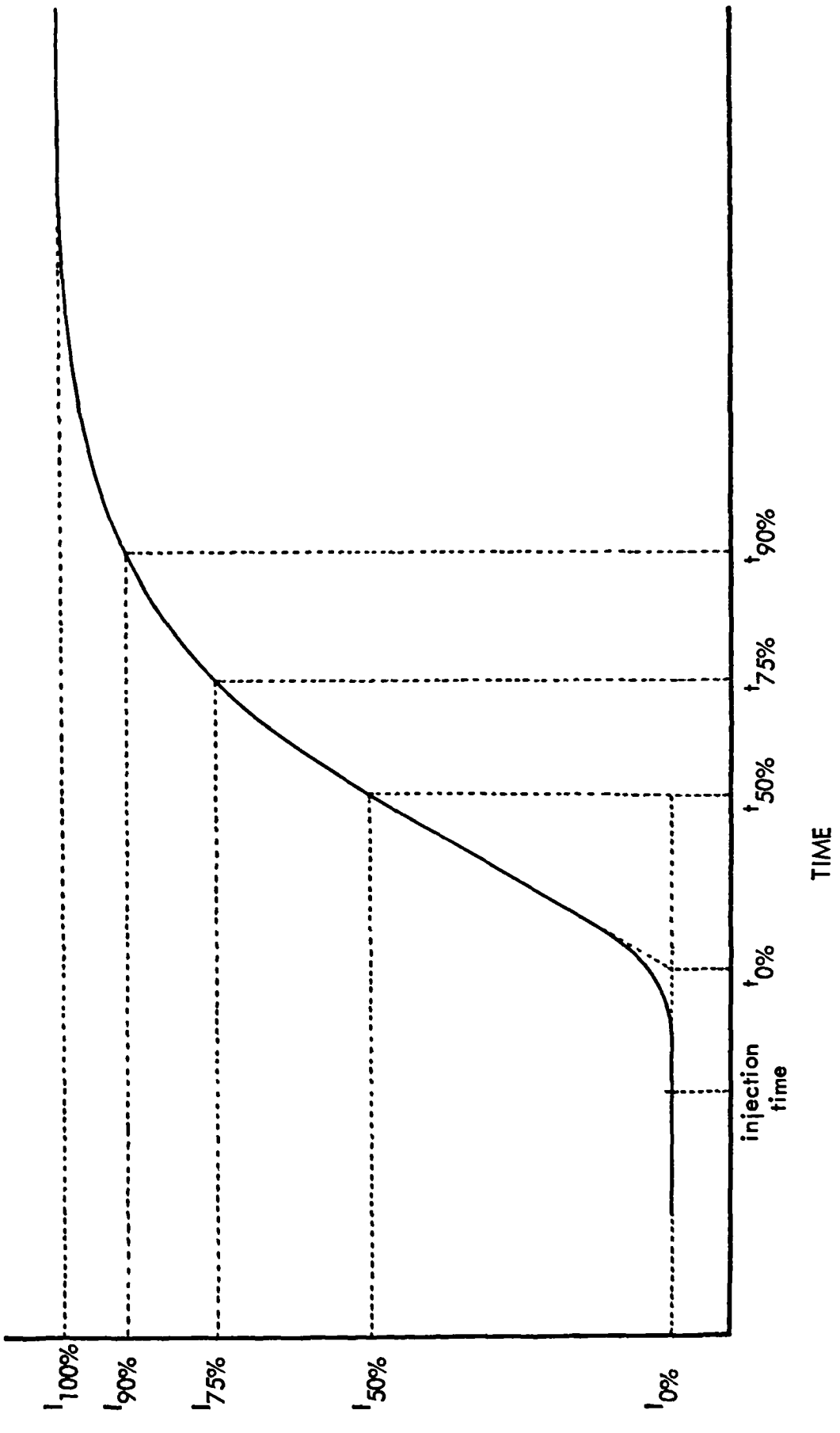
Parameters were expressed as means and standard deviations of five replicates of each experiment.

3.2.5.2 Assessment of emulsion quality

Introduction

The quality of emulsions can be compared using two parameters; the mean particle size of the emulsion and the width of the size range. A wide variety of emulsions were examined during the study and required two techniques to assess their relative quality. Mean particle size was measured using a Coulter Nanosizer and checked by estimation using light microscopy. When use of the Nanosizer

Figure 3.1 General shape of curve for evaluation of emulsification rate.



was inappropriate, microscopy was used to estimate the size of coarse emulsions and to judge the width of size ranges by observing the number of large particles.

3.2.5.2.1 Determination of mean particle size using a Coulter Nanosizer

Briefly, the Coulter Nanosizer exploits fluctuations in intensity of scattered laser light due to Brownian motion of dispersed particles. The intensities themselves are not measured but the time between fluctuations is correlated for many particles in the bulk dispersion. Each time span is a function of particle size and the viscosity of the continuous phase. Thus the mean particle size can be computed together with a 'polydispersity index'; a measure of size range calculated from the spread of the data. The instrument can measure mean particle size for particles in the range 40-3000nm and presents the polydispersity index as a score between 0 and 9 (narrow to wide size range: monodisperse to a width range of about 9 to 1 by diameter[156-157]). Emulsions were transfered from the self-emulsification cell to Coulter Nanosizer tubes by means of a glass pipette. Data was correlated for two minutes for each size determination and experiments were conducted in triplicate.

3.2.5.2.2 Examination of emulsion stability using a Coulter Nanosizer

Certain emulsions were sealed in Coulter Nanosizer tubes using an ampoule sealing device and stored at controlled temperature. The stability of these emulsions was estimated by measuring the mean particle size (using a Coulter Nanosizer) at various storage times.

3.2.5.2.3 Microscopy

A sample of each emulsion was examined by light microscopy using a haemocytometer with calibrated grid. Emulsions were allowed to settle for fifteen minutes. In each case photographs of three 'fields of view' were recorded.

3.2.6 Physical properties of homogenised systems

Homogenised emulsions, identical in composition to self-emulsified systems, were prepared under controlled conditions using a Silverson homogeniser. Particle sizes were determined in triplicate and stability assessed using a Coulter Nanosizer as described above.

3.2.7 Assessment of affinity of Tween 85 for aqueous phases

Tween 85 exists in solution, or micellar solution, in water up to a concentration of $10^{-4}\%^{w}/v$ (approximately 5×10^{-7}M). Above this concentration, depending on the presence of other dissolved materials, phase separation begins to occur. The precise concentration at which phase separation occurs is difficult to determine.

Phase separation is difficult to detect because, even when it is unable to exist in thermodynamically stable solution, Tween 85 exists as a highly metastable colloid when mixed in water. It is probably more sensible to accept the description of a phase separation region for such surfactants particularly as the materials are mixtures of molecules of varied molecular structure.

Dispersions containing $10^{-1}\%^{w}/v$ T85 were prepared using a Silverson homogeniser. One litre of each mixture was homogenised for fifteen minutes in a glass two litre vessel. Dilutions of the stock dispersions were prepared and each homogenised for a further fifteen minutes (200ml mixtures in a 400ml glass vessel). Samples of each dispersion were allowed to equilibrate for twenty-four hours at 25 $\pm$ 0.5°C. The relative intensity of light scattering was measured in triplicate for each dispersion and for pure continuous phases using a Laser Nephelometer PD2 (Travenol Laboratories Ltd.). In short, a sample contained in a glass cell was placed in a helium-neon laser beam (λ = 632.8nm) and the instrument measured the intensity of light scattered (on an arbitrary scale) forwards at an angle of 31° to the incident beam. Relative scattering intensity due to Tween 85 particles ($I - I_o$) was calculated by subtracting scattering intensities of pure continuous phases (I_o) from values for corresponding dispersions (I).

3.2.8 Photomicrographic study of the emulsification process

It was of interest to consider whether any differences could be seen in the dynamic behaviour of different oil-surfactant mixtures brought into contact with water. A simple but effective technique was useful. A droplet of water was placed onto the surface of a clean, glass microscopy slide. A drop of oil-surfactant mixture was placed adjacently and allowed to spread towards the water droplet. As the material was spreading the microscope was focused on the region in which the two surfaces would inevitably meet allowing observation of disruption of the interface. Following the initial interaction emulsification ceased due to a lack of an excess of water. Subsequently textures formed by interaction of the two phases could be observed. Photographs were recorded for comparison.

3.3. BIOPHARMACEUTICAL METHODS

Development of the assay procedure for benzoic acid metabolites in urine is described in Appendix Two. Hippuric and benzoic acids were assayed by ultraviolet spectrophotometry following high-pressure liquid chromatography of diluted urine. The assay procedure was quick and results were precise; laborious extraction or derivitization procedures were avoided, improving on published assay methods.

3.3.1 Dietary restrictions

Hippuric acid appears in normal urine as a metabolite of quinic acid digested from fruit and some vegetables. The metabolism of quinic acid is relatively slow. For this reason subjects were asked to avoid fruit and vegetable for twenty-four hours prior to and during each experiment. Subjects fasted on appropriate mornings and for at least four hours after benzoic acid administration.

3.3.2 Fluid intake

Excretion rate may be affected by fluid intake. In addition stomach emptying (and therefore absorption rate) is likely to be affected by fluid intake. Therefore a standard fluid intake was desirable. Sufficient water was required to ensure that most excreted material was evacuated from the bladder at each collection time. (When the volumes of urine passed were less than 50ml significant amounts of metabolite remained in the

bladder). Fluid intake was standardized by requesting subjects to drink 200ml of water each half hour commencing 90 minutes before benzoic acid administration. Two hours after administration the fluid intake was reduced to 200ml per hour.

3.3.3 Urine samples

Subjects discarded morning urine but noted the time of urination. Subsequently urine was collected at -90, -15, zero, 15, 30, 45, 60, 90, 120, 150, 240, 360 and 480 minutes with respect to the time of benzoic acid administration. The volume of urine collected was recored in each case and samples retained for assay.

3.3.4 Formulations

250mg benzoic acid was administered in each of three formulations (contained in size 000 hard gelatin capsules) to each of six healthy, male subjects. Two additional formulations were administered to three of the subjects. The five formulations and their significance are tabulated below.

FORMULATION	SIGNIFICANCE
1. 250mg powdered benzoic acid	powdered drug
2. 5g (5%^{W}/w benzoic acid solution in Miglyol 812)	representative of a non-emulsifying oil solution
3. 5g (5%^{W}/w benzoic acid solution in 70% Miglyol 812/ 30% Tween 85)	representative of an efficient self-emulsifying formulation
4. 5g (5%^{W}/w benzoic acid solution in Tween 85)	representative of a slowly emulsifying formulation
5. 250mg powdered benzoic acid PLUS 5g (70% Miglyol 812/ 30% Tween 85)	an experiment to determine whether mere presence of surfactant affected absorption of benzoic acid

CHAPTER FOUR

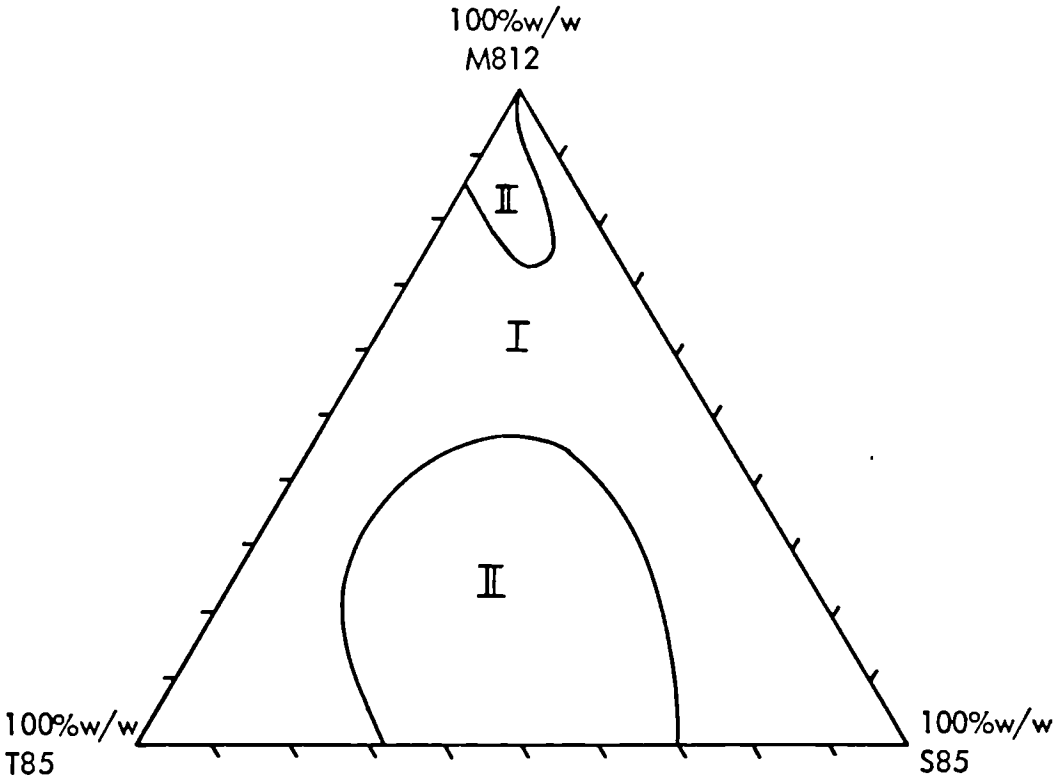
RESULTS AND DISCUSSION

4.1. THE BEHAVIOUR OF M812/T85/S85 MIXTURES IN DISTILLED WATER

4.1.1 Phase behaviour, viscosities and densities of oil/surfactant mixtures at equilibrium.

The phase diagram for the tertiary mixture Miglyol 812 (M812), Tween 85 (T85) and Span 85 (S85) at ambient temperature was, apparently, that of a mixture of two pairs of partially miscible liquids. M812 and S85 were miscible in all proportions but the other two pairs (T85 and S85, M812 and T85) produced diphasic mixtures over wide ranges of composition. At these compositions two phases separated out, one rich in either material. Thus the ternary phase diagram (Fig 4.1.) showed two diphasic regions, one radiating outwards from the T85/S85 axis, the other from the M812/T85 axis. The M812/T85 diphasic area was of particular interest. It appeared that T85 was poorly soluble in M812 ($<1\%^{w}/w$) but that T85 could solubilize large amounts of M812. Infact this was shown to be behaviour conferred on the mixture by the presence of water in the industrial materials. The T85 had a moisture content of $5.0\%^{w}/w$ (measured by the Karl-Fischer method) as waters of hydration associated with the oxyethylene chains. This represented 5.3 water molecules for each average T85 molecule and was typical of the water content of the industrial material. The water content of four separate batches ranged from 4.8%to $5.1\%^{w}/w$. It was difficult to dry the surfactant materials possibly due to the fact that the water would have been hydrogen bound to the ether oxygen atoms of the oxyethylene chain.

Figure 4.1. Phase diagram of the industrial grade materials Miglyol 812 (M812), Tween 85 (T85) and Span 85 (S85) at 25°C.



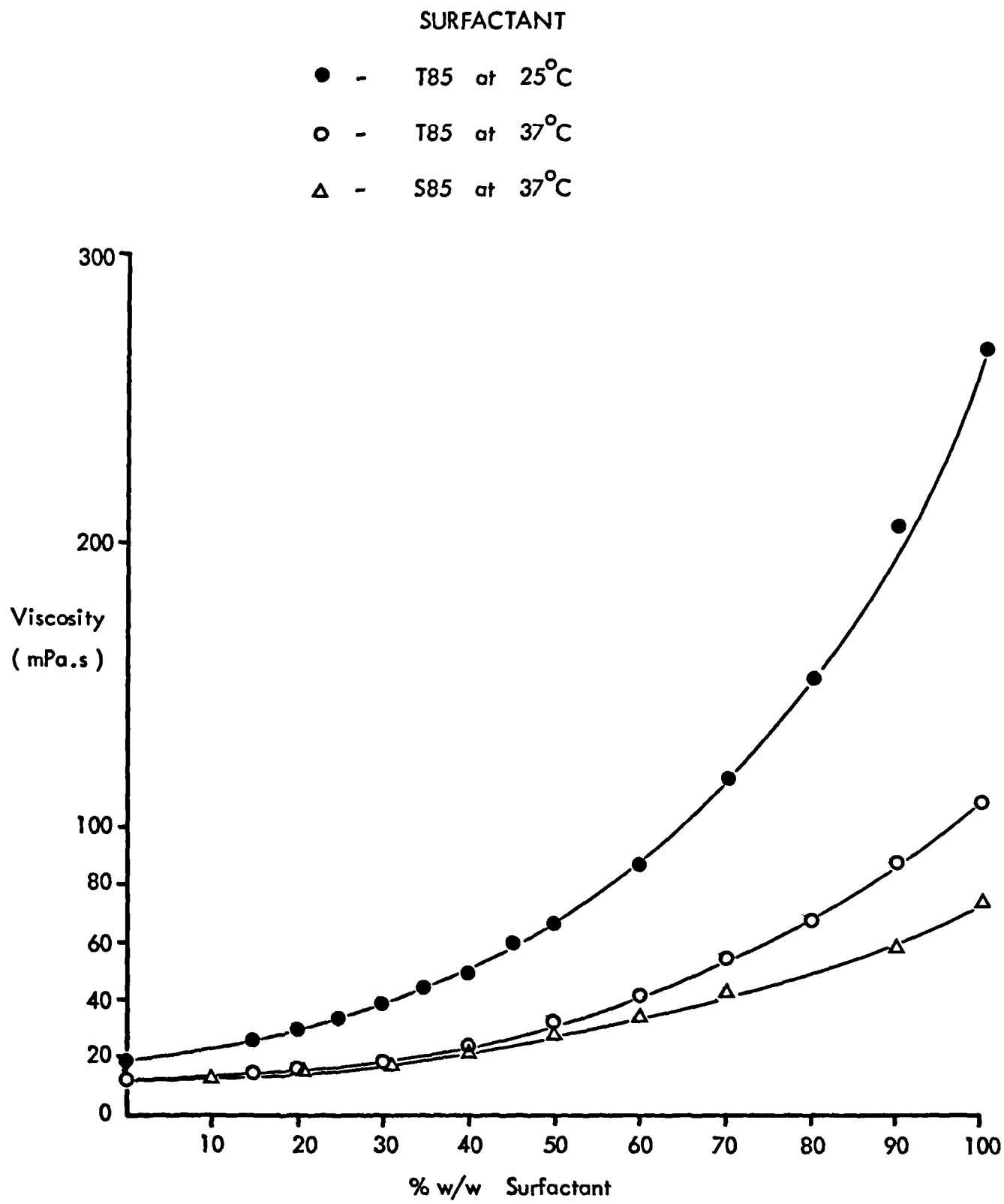
I - monophasic mixtures

II - diphasic mixtures

In addition, water is thought to be trapped within the polyoxyethylene chains which exist in a helical conformation in the presence of water[110,158-160]. However by repetitive partitioning with molecular sieve material (BDH Molecular Sieve Material 4A) the water content of T85 was reduced to 0.34%^{w}/w (0.36 water molecules per molecule of T85). When M812/T85 mixtures were prepared using the dried material the diphasic region disappeared, as did that caused by T85/S85 mixtures. In the absence of water, the three materials polyoxyethylene-(20)-Sorbitan trioleate, sorbitan trioleate and Miglyol 812 were mutually soluble. It is reasonable to assume that when small amounts of T85 were added to M812 the water content of the M812 (0.1%^{w}/w by the Karl Fischer method) was sufficient to cause phase separation of the surfactant material.

Viscosities of M812/surfactant mixtures were measured up to shear rates of 1663 sec^{-1} using a cone and plate viscometer. All mixtures were Newtonian up to this shear rate over the temperature range 25-37^{o}C. The behaviour of viscosities when plotted against mixture composition deviated consistently from ideality. This was thought to be due to moisture content as other authors have suggested[38,161]. Figure 4.2. illustrates typical behaviour of M812/surfactant mixtures. M812/T85 mixtures were the most viscous. The use of S85 as an alternative surfactant lowered the viscosity and reduced the deviation from ideality (due to the lower water content

Figure 4.2. Viscosities of Miglyol 812/surfactant mixtures.



- 0.3%^{w}/w - of the S85). Viscosities for oil/surfactant mixtures using mixtures of T85 and S85 as the surfactant fell between those of mixtures using each surfactant alone. Increase in temperature lowered viscosities but did not reduce the deviation from ideality.

Figure 4.3. represents corresponding plots of densities of M812/surfactant mixtures. These plots deviated slightly from ideality although the effect was not as marked as that on viscosities. The difference between densities of S85 mixtures and T85 mixtures was large but changes in temperature did not have such prominant effects. Temperature affected the value of viscosities much more profoundly.

It is reasonable to speculate that deviations from ideal behaviour of mixture viscosities and densities may have been caused by micellization within the non-aqueous medium. Micellization in anhydrous non-aqueous solvents has been described although the free energy changes favouring such associations are small in comparison with those of aqueous micellization[162]. However in the presence of water hydrogen bonding between water and ether oxygens in T85 could promote micellization[163]. Using the dried sample of T85 as a comparison with the raw material the latter reasoning seemed justified. Both densities and viscosities were reduced using the dried T85 (Fig. 4.4.) and the deviation from ideality of the viscosity plots was

Figure 4.3. Densities of Miglyol 812/surfactant mixtures.

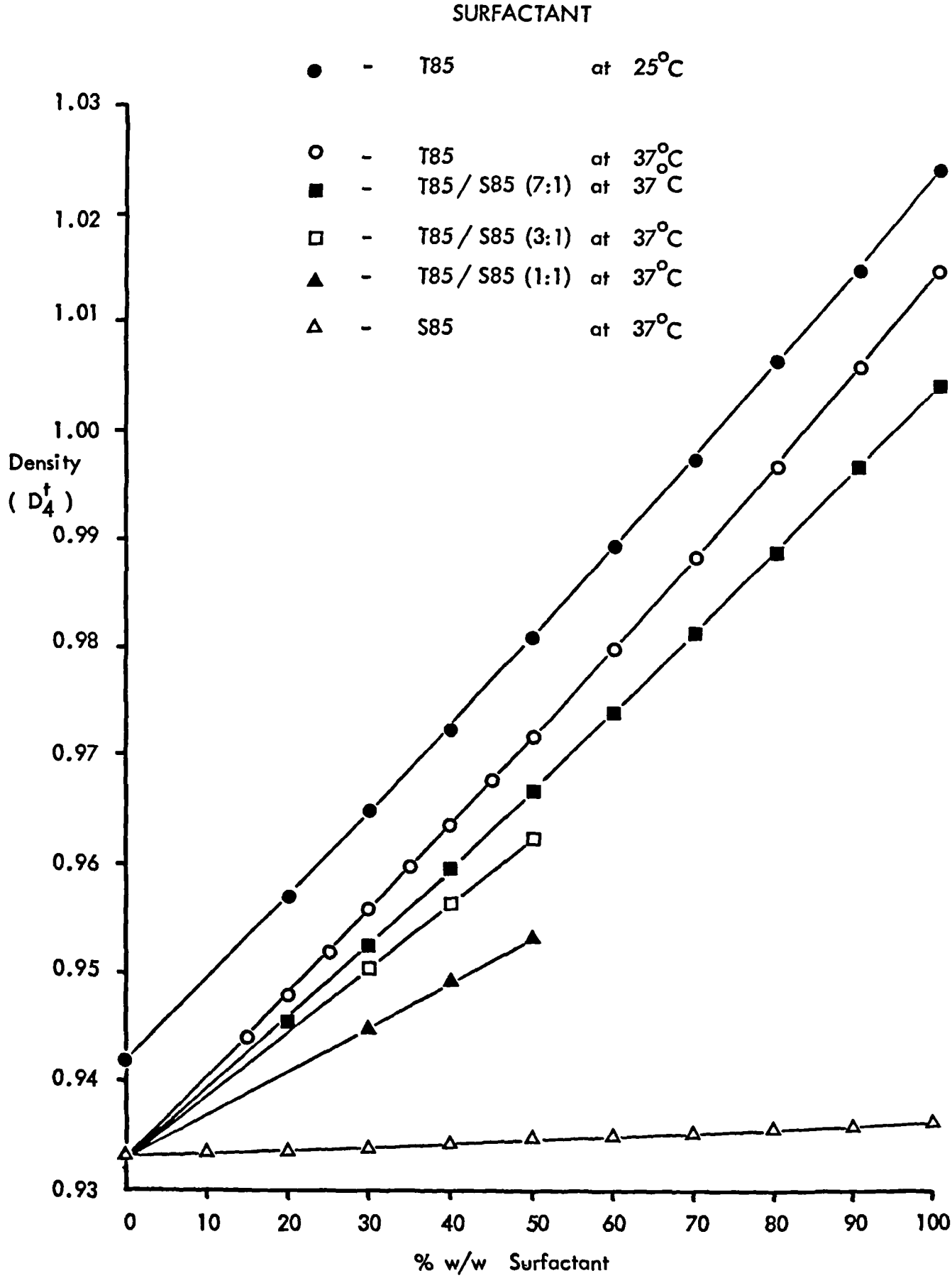
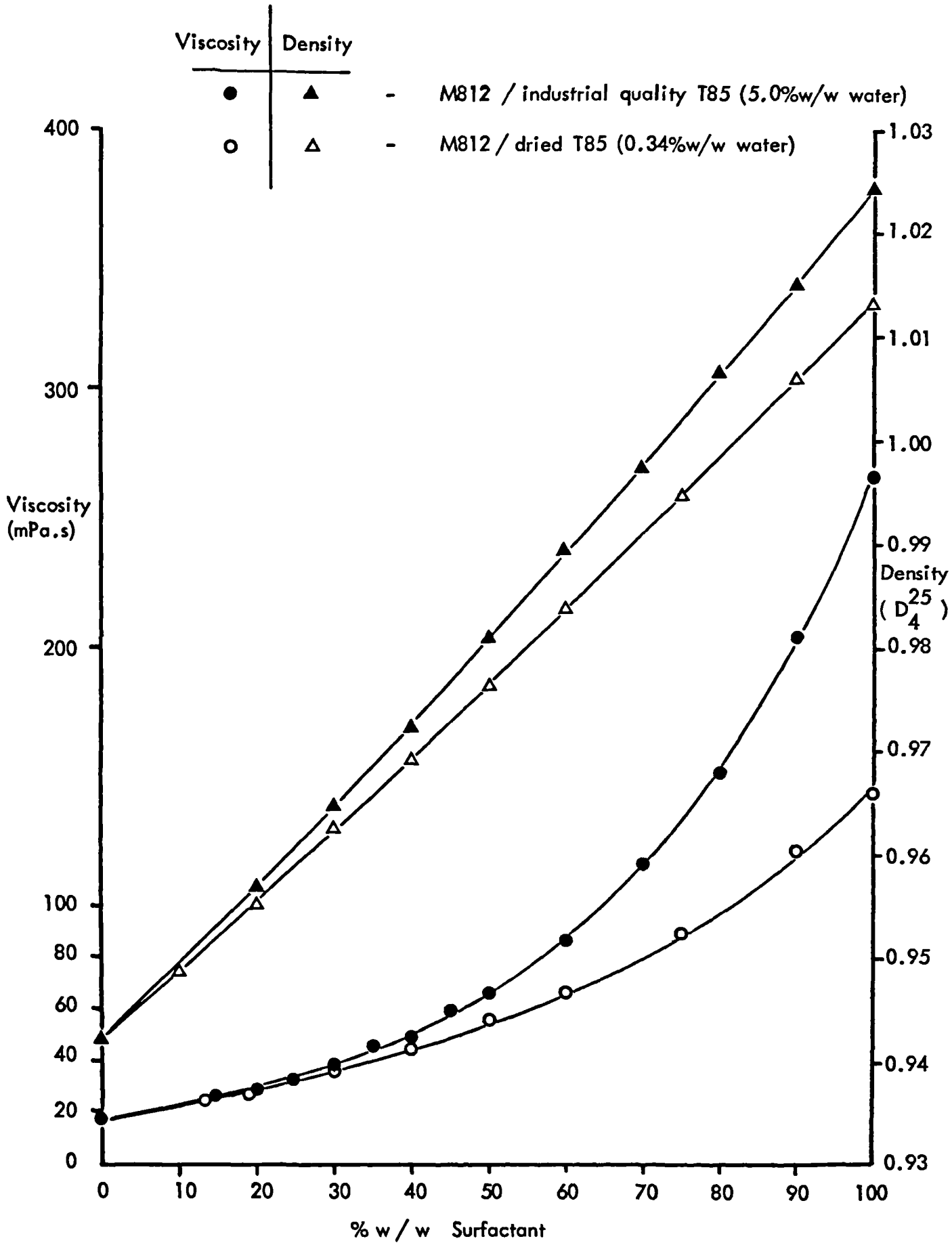


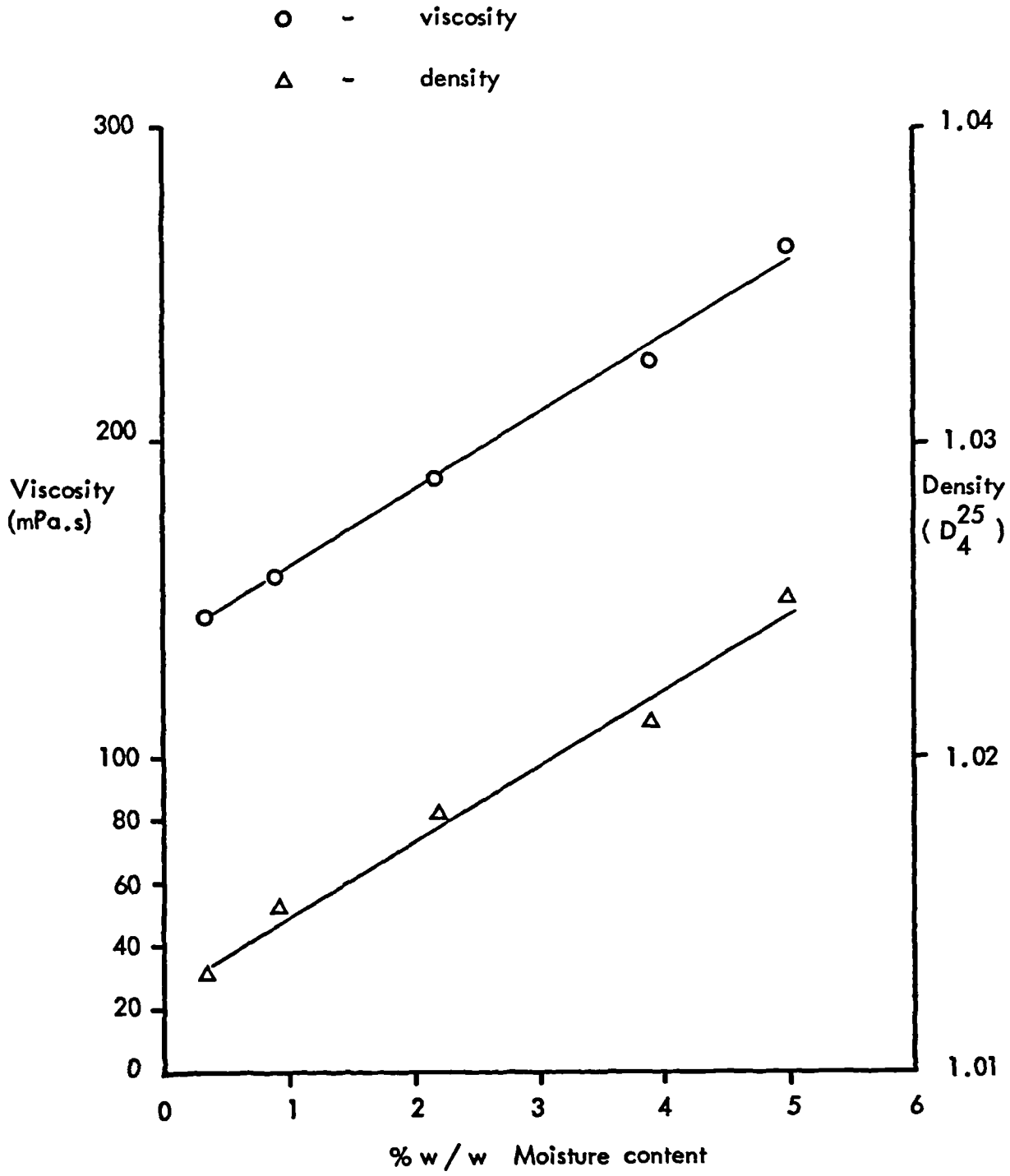
Figure 4.4. Effect of moisture content on viscosities and densities of M812/T85 mixtures at 25°C.



significantly reduced. This suggests that micellization of T85 within the M812 was responsible for non-ideal behaviour and that if the material could have be dried completely, the mixtures may have behaved ideally. Figure 4.5 shows that the viscosity and density of T85 increased steadily, near to linearly, over the range 0.34 to 5.0%^{w}/w water content, as the extent of ordered association increased.

In view of the influence of moisture content on the properties of the surfactants, the industrial materials will be referred to as T85, T80, T81 and S85. The corresponding anhydrous surfactants will be referred to as POE-(20)-S-trioleate, POE-(20)-S-monooleate, POE(5)-S-monooleate and S-trioleate.

Figure 4.5. Effect of moisture content on viscosity and density of POE-(20)-S-trioleate at 25°C.



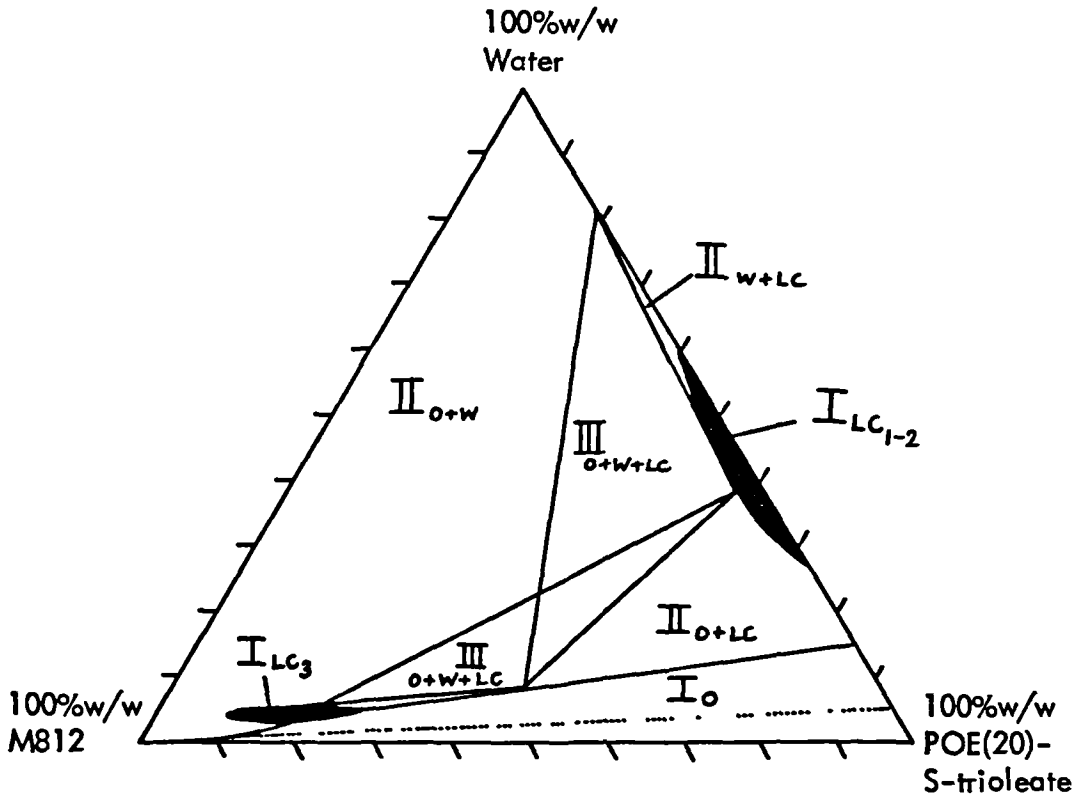
4.1.2 Phase behaviour of hydrous mixtures at equilibrium

Phase behaviour of nonionic surfactant/oil/water systems has been studied extensively over recent years[69,109,112,164]. The phases present have been shown to be similar to those described for ionic surfactant systems by previous authors[152-153, 165-168]. In nonionic systems the effect of temperature may be particularly pronounced as highlighted by the comprehensive, elegant work of Shinoda's school[112].

During the current study the following convention is used to denote phase behaviour. Roman numerals I, II, III represent the number (one, two, three etc.) of distinct thermodynamically stable phases which exist in equilibrium in each particular mixture. The suffixes w, o, LC (water, oil, liquid crystal) are used to denote the predominant solvent of each phase. For example, II_{w+o} describes a mixture consisting of two phases one water-rich, the other oil-rich, although both phases may contain dissolved surfactant and solubilized material. Thus if a third phase of liquid crystalline material exists in equilibrium with oil and water phases the phase behaviour is described as III_{w+o+LC}. When three phase mixtures are predominantly liquid crystal the mixture is described at III_{LC+w+o}.

As water was added to binary mixtures of M812 and POE-20-S-trioleate at 25°C solubilization took place until the 'inverted' micelles became saturated. This saturation point denoted the phase boundary of the isotropic oil phase, region I_o (Fig. 4.6.). At higher water contents large areas of the phase diagram involved liquid crystalline phases although the regions of clear liquid crystalline mixtures were small. At low M812 contents a region of clear liquid crystal was found close to the surfactant/water axis. This region appeared to consistof two liquid crystalline types, LC_1 and LC_2, which had different appearances under polarised light. X-ray diffraction studies were not available but it is reasonable to assume that the LC_1 and LC_2 were of the Lamellar and Hexagonal type as described by, for example, Rosavear[153]. Large areas of the phase diagram were mixtures of water-rich, oil-rich and liquid crystalline phases (region III_{w+o+LC}). At lower surfactant contents the liquid crystal was not detected as a bulk phase and mixtures consisted of water-rich and oil-rich phases (region II_{w+o}) many of which were metastable emulsions of one phase in the other. These were oil in water emulsions except when the oil/water volume ratio was high. One other important phase which formed in the presence of small proportions of water was a third region of clear liquid crystal (I_{LC3}). This was a fluid phase with no appreciable elasticity (c.f. LC_1 and LC_2 regions) and was probably a 'reversed hexagonal' phase. This phase is not common in nonionic systems[164] and generally occurs for surfactants which

Figure 4.6. Phase diagram of M812, POE-(20)-S-trioleate and water at 25°C.



do not form aqueous micelles[169]. This is consistent with the properties of POE(20)-S-trioleate which did not form aqueous micelles at 25°C. Thus an isotropic water phase (I_w) was not found. The dotted line running from the M812 apex through the I_o phase represents the composition of M812/T85 mixtures using the industrial materials, which include water as described in section 4.1.1.

Figure 4.7. illustrates the phase diagrams for M812/POE-(20)-S-trioleate/S-trioleate mixtures with fixed water content between 0 and 10%^{w}/w. The diagrams show that phase behaviour was more sensitive to POE-(20)-S-trioleate concentration than the lipophilic S-trioleate. Liquid crystalline phases were formed in the presence of S-trioleate but were diminished as the concentration of liphophilic surfactant increased above about 10%^{w}/w.

Figures 4.8-4.16 represent a more detailed study of M812/T85/water phase behaviour conducted at different temperatures. The correlation of these phase diagrams with emulsification properties will be discussed later.

As water was added to T85 (Figure 4.8) the water was solubilized to form a single surfactant phase (I_s) until eventually the surfactant was saturated. A lamellar liquid crystalline phase began to separate out which existed in equilibrium with the surfactant phase to form the

Figure 4.7. Phase behaviour of some M812, POE-(20)-S-trioleate, S-trioleate and water mixtures at 25°C.

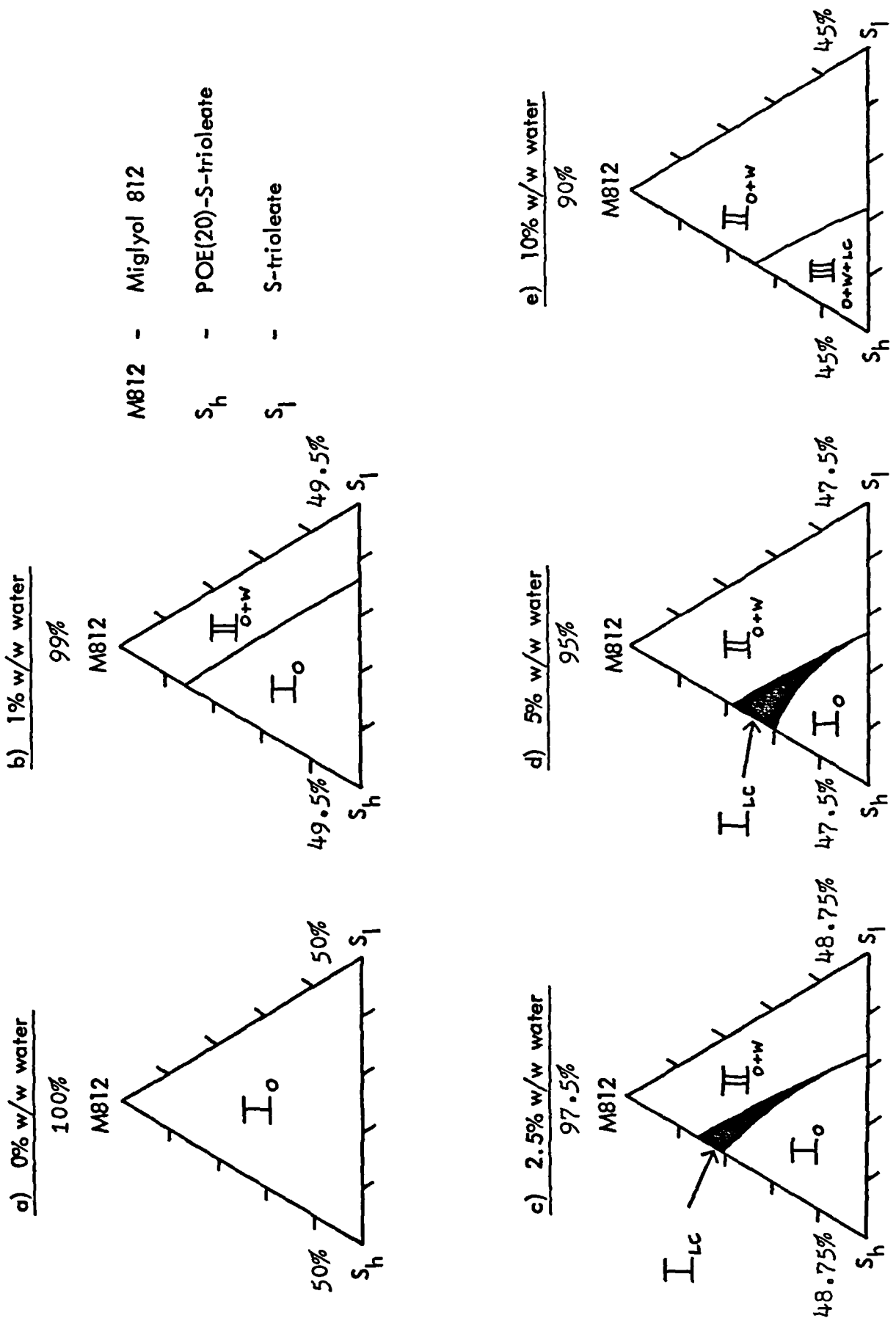


Figure 4.8. Phase diagram of water and POE-(20)-S-trioleate

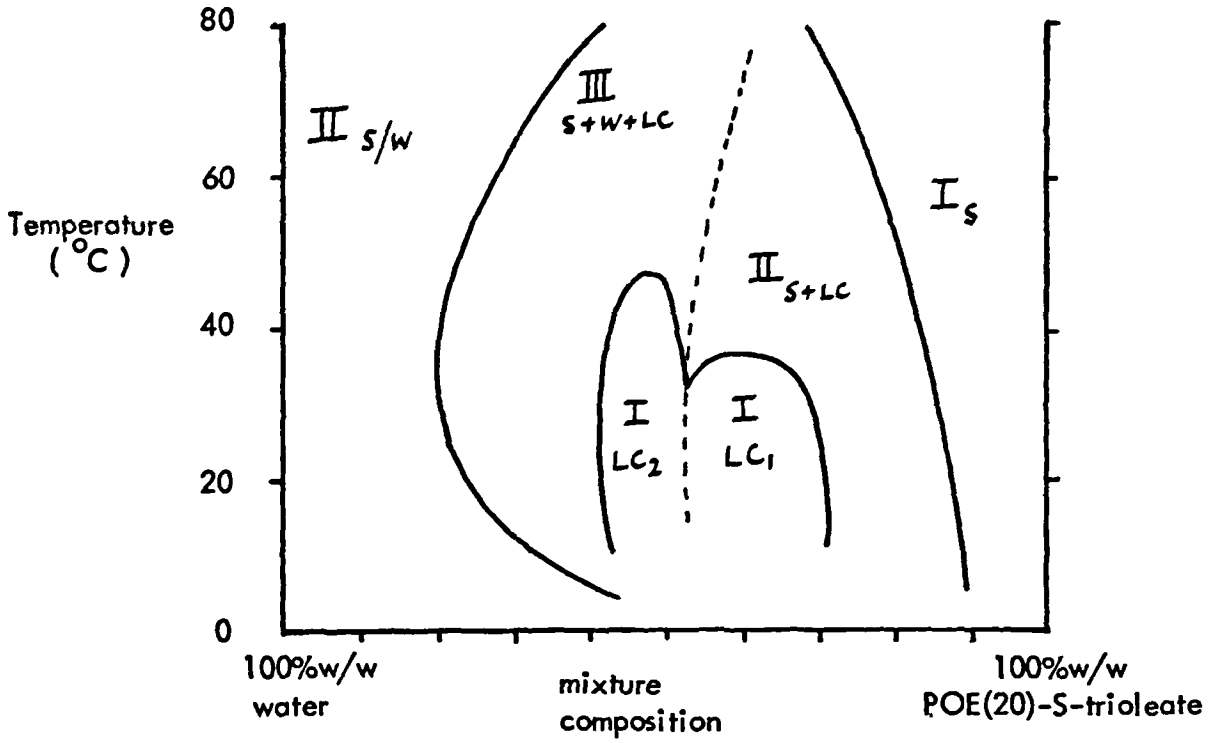
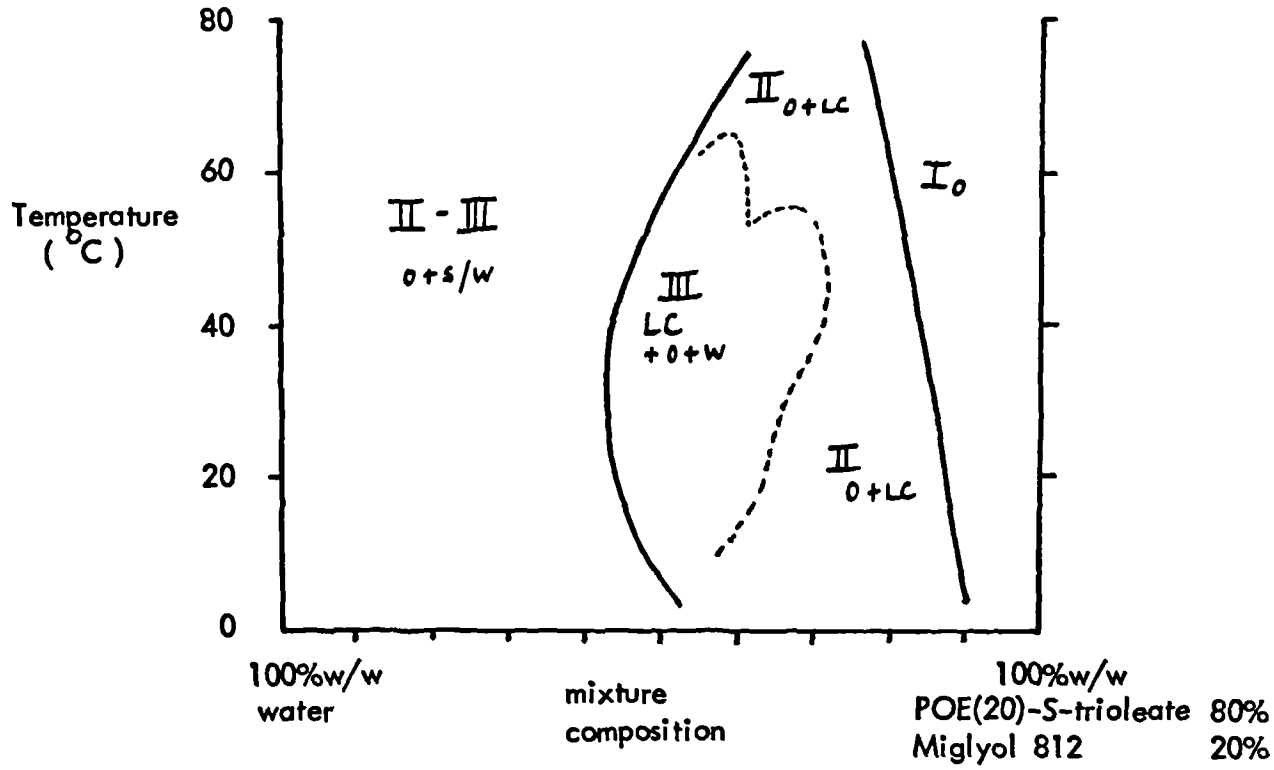


Figure 4.9. Phase diagram of water and the mixture 80%^{w}/w POE-(20)-S-trioleate and 20%^{w}/w M812.



biphasic system II_{S+LC}. The effect of temperature, between 0 and 80°C, on the I_S phase was to increase the capacity for water solubilization. A region of enhanced water solubilization, as investigated by Shinoda and co-workers[118,120,124], was not found between 0 and 80°C. At higher concentrations of water clear anisotropic phases existed below approximately 35°C. Above this temperature liquid crystalline phases existed in equilibrium with other phases. If water concentration was further increased a dispersion of T85 in water resulted. As mentioned previously, T85 was insufficently hydrophilic to form a micellar solution in water and formed a metastable dispersion of larger aggregates. Such behaviour corresponds to the behaviour of surfactants of similar hydrophile-liophile balance (H.L.B.) in Shinoda's range of polyoxyethylenated nonylphenylethers.

Figures 4.9 to 4.16 represent corresponding phase diagrams for various water and oil/surfactant mixtures. The addition of 20% oil to the surfactant phase, in the surfactant/oil ratio of 80/20, resulted in considerable modification to the water-surfactant phase diagram. In Figure 4.9 the extent of the area containing liquid crystalline phases is much reduced and the monophasic liquid crystalline systems were not formed. However some mixtures (III_{LC+o+w}) were substantially liquid crystalline. Further addition of M812 to the system (Fig. 4.10) reduced the liquid crystalline region and the capacity for

Figure 4.10. Phase diagram of water and the mixture 60%w/w POE-(20)-S-trioleate and 40% M812.

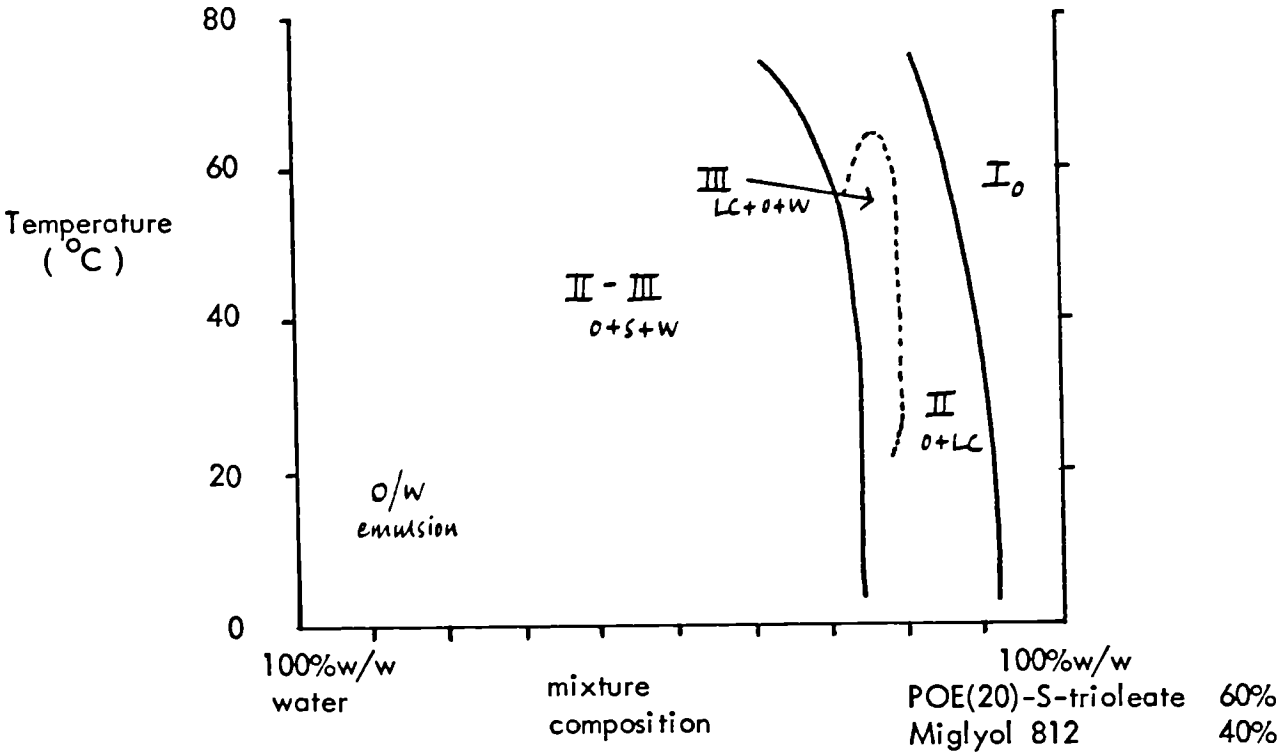
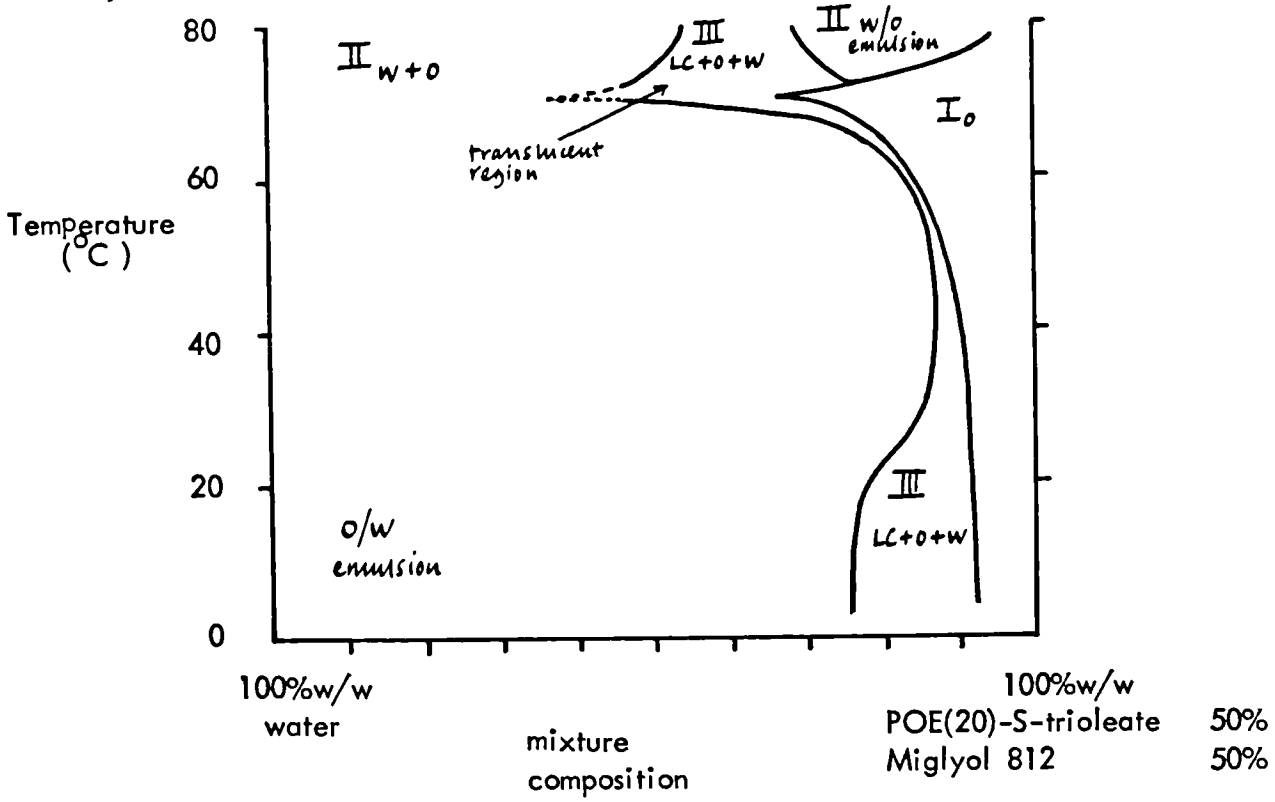


Figure 4.11. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% M812.



solubilization of water in the oil phase (I_o region).

At a surfactant/oil ratio of 50/50 a region of high water solubilization appeared at approximately 70°C. An equivalent region may have occured above 80°C at lower M812 contents. Generally, the presence of increasing amounts of oil tended to lower the temperature at which such regions of solubilization occured but also reduced their size (as in Shinoda's experiments[112]). Mixtures which had compositions fractionally outside the regions of critical water solubilization had a translucent quality and contained high proportions of birefringent material (III_{LC+o+w}). Similar mixtures have been described by Friberg's[109] and Shinoda's[137] Schools and have been explained in terms of a 'surfactant phase' which includes large amounts of solubilized water and oil. Shinoda has proposed that such mixtures may exist in a laminar form. Above a critical temperature water was no longer solubilized within the surfactant phase and a water-in-oil emulsion resulted. (II_{w+o} - denoted as $II_{w/o}$). The region of critical water solubilization described above was a phase inversion region which occured at a characteristic temperature similar to the Phase Inversion Temperature (PIT) of emulsions as discussed in section 2.4. However true phase inversion, defined as an inversion from oil-in-water to water-in-oil emulsion 'type', did not occur at low volume fractions of water.

Oil/surfactant ratios greater than 50:50 were particularly important with respect to emulsification properties. Their phase behaviour is compared to the 50:50 ratio on an expanded scale in Figures 4.12 to 4.16. The size of the I_o region decreased as the oil/surfactant ratio increased. At the same time the region of phase inversion occured at lower temperatures. At ratios higher than 85%M812/15%T85 the region I_o was extremely small and a critical region of solubilization could not be detected. Between 50%M812/50%T85 and 80%M812/20%T85 solubilization regions could be identified progressively decreasing in size and occuring at lower temperatures (i.e. the critical temperature was lowered as the proportion of M812 increased). Figures 4.14 and 4.15 differ in one important way from the other phase diagrams in that they show a clear liquid crystalline phase adjacent to the I_o phase. This phase has been identified previously in Figure 4.6 and probably represented an 'inverse hexagonal' phase. The latter phase is important since in certain circumstances it represented a single phase formed on addition of water to the saturated I_o phase. In other circumstances such as a) in Fig. 4.14 above 60°C b) in Fig. 4.15 above 30°C or c) in Fig. 4.16, when water met the I_o phase above the critical solubilization temperature (CST), a two phase mixture had to result. Alternatively if one considers water meeting the I_o phase well below the CST in cases such as a) in Fig. 4.13 below 20°C b) in Fig. 4.12 below 40°C c) in Figs. 4.8 to 4.10

below 80°C, then one would expect the separation of a lamellar type liquid crystal to result, again, in a two phase system.

Figure 4.12. Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% M812 (expanded scale).

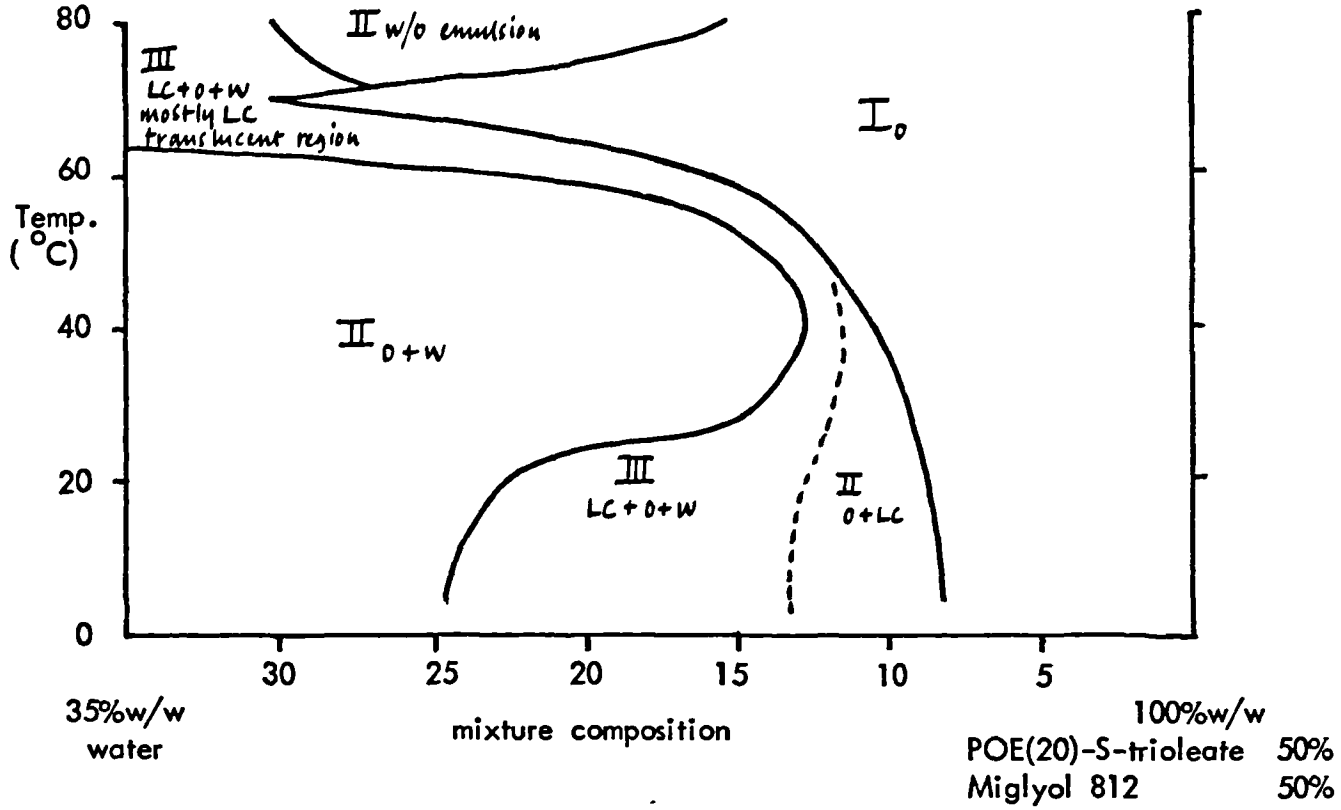


Figure 4.13. Phase diagram of water and the mixture 40% POE-(20)-S-trioleate and 60% M812.

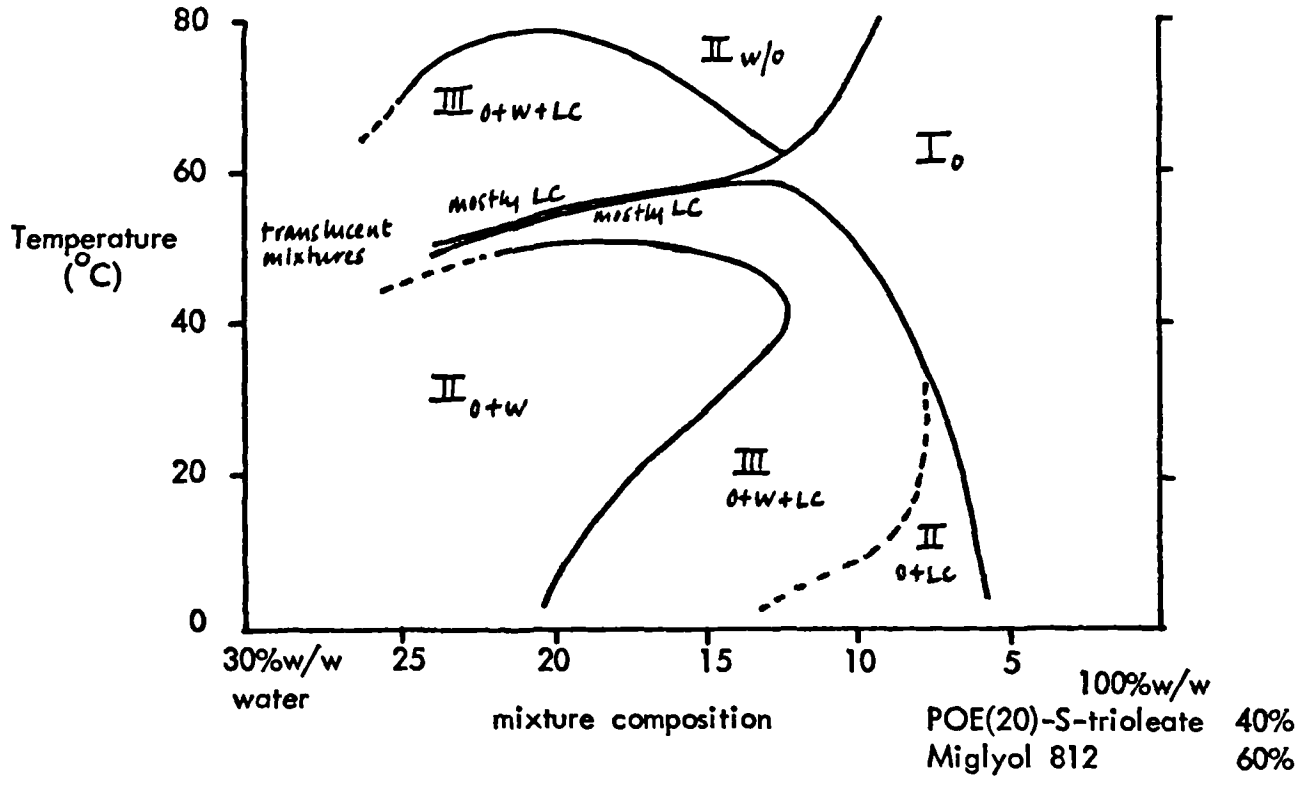


Figure 4.14. Phase diagram of water and the mixture 30% POE-(20)-S-trioleate and 70% M812.

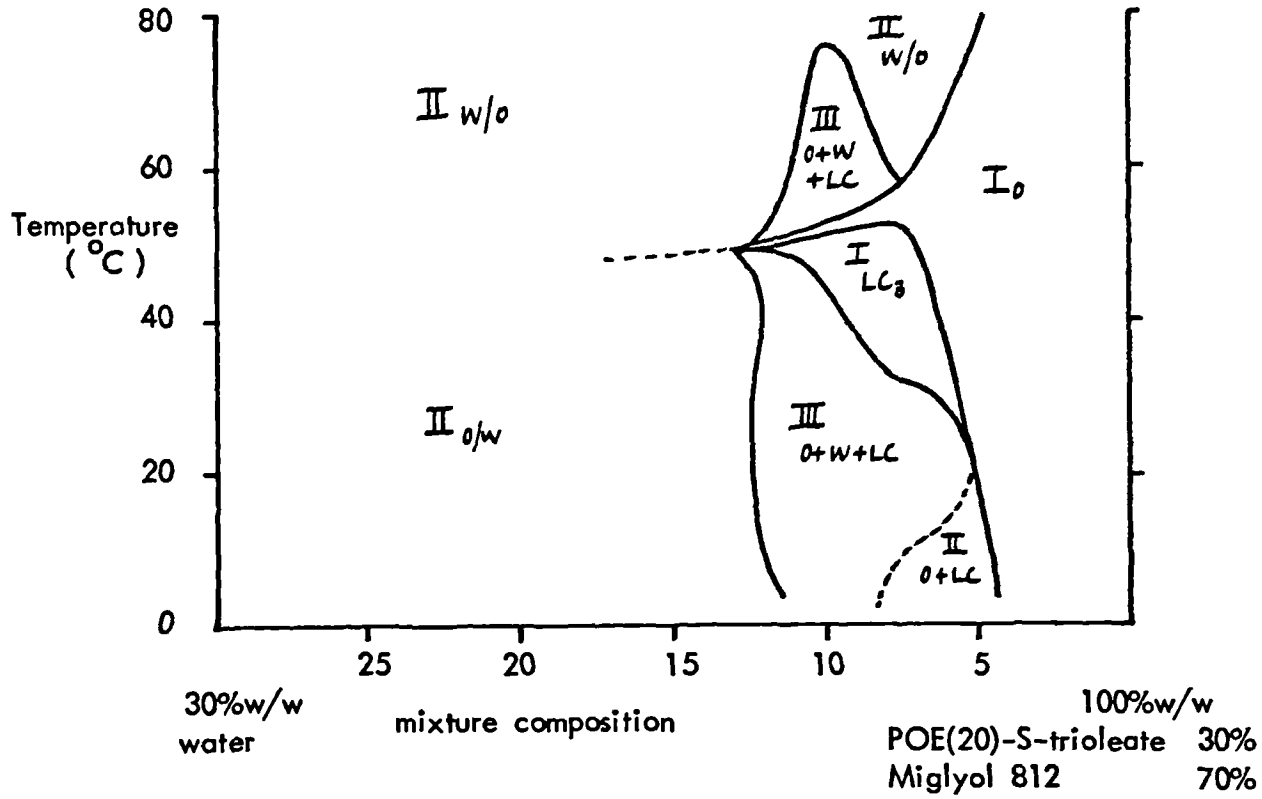


Figure 4.15. Phase diagram of water and the mixture 20% POE-(20)-S-trioleate and 80% M812.

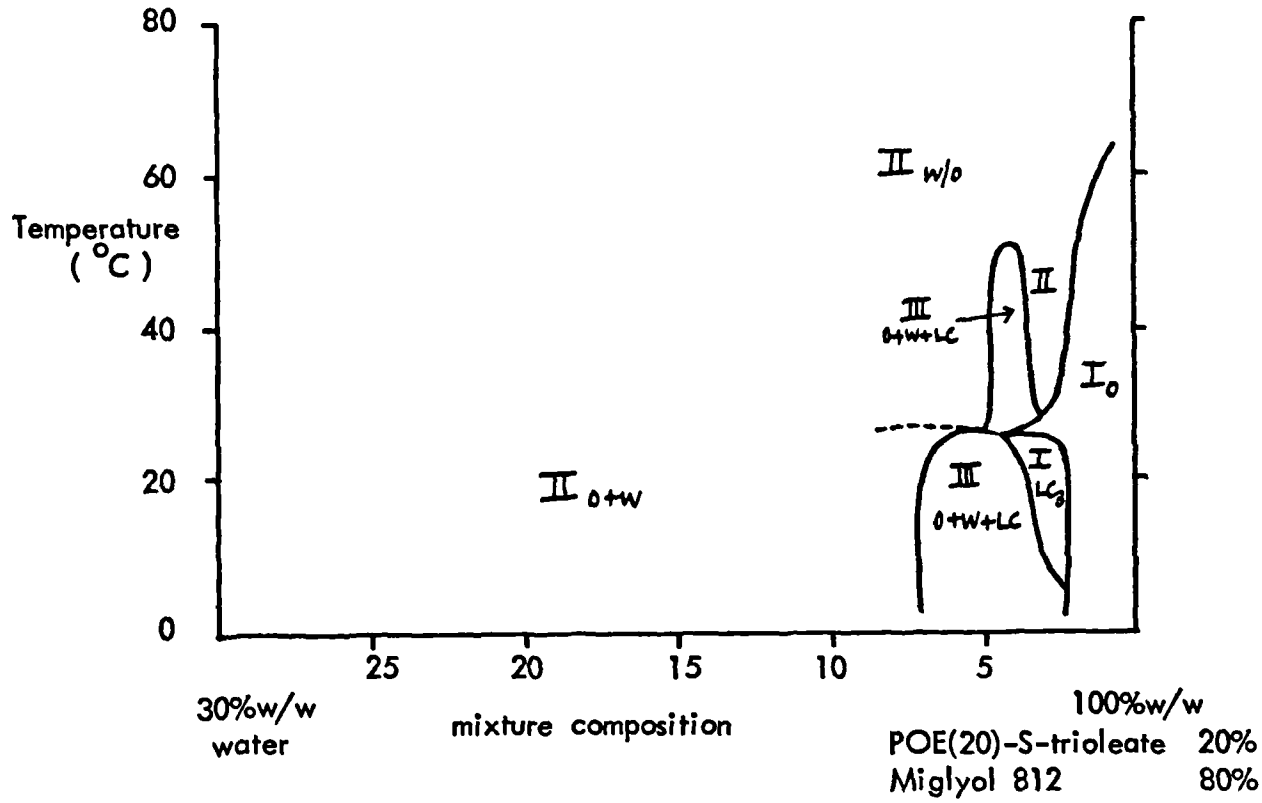
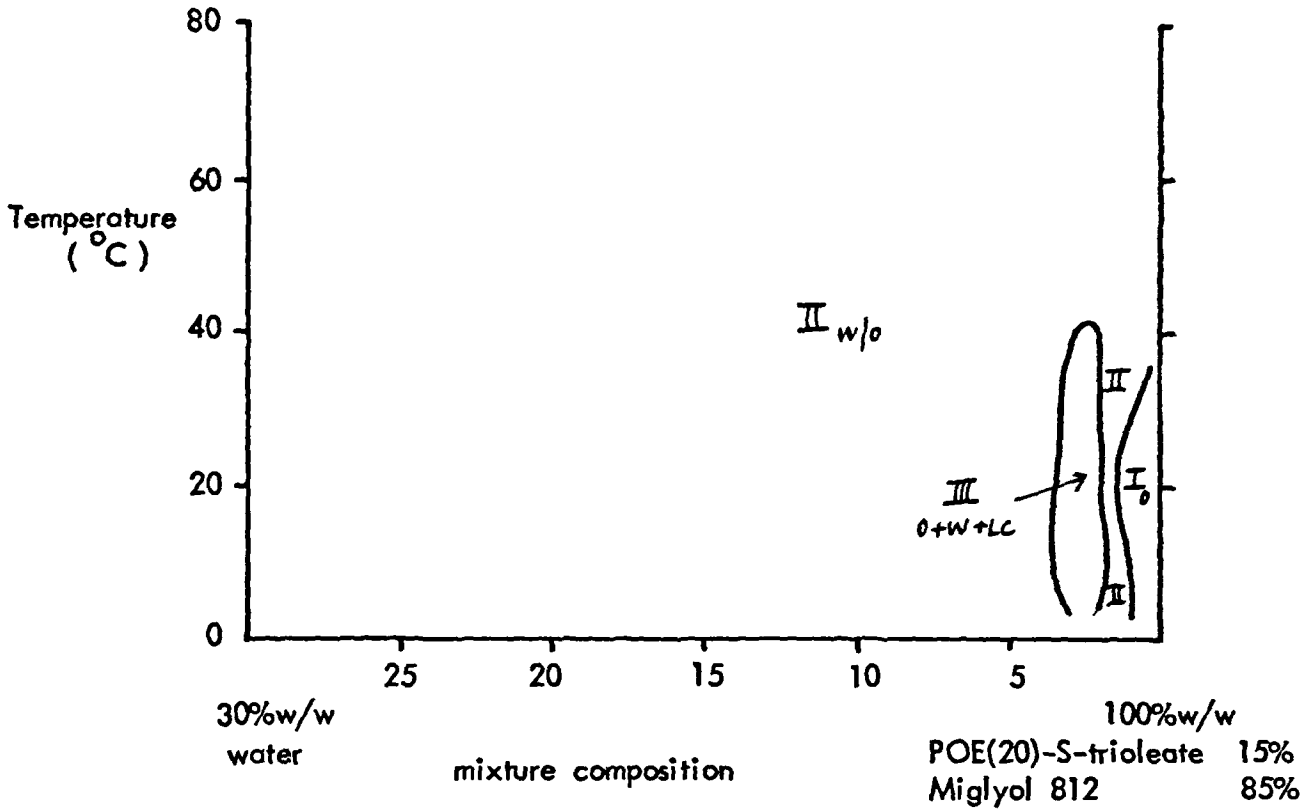


Figure 4.16. Phase diagram of water and the mixture 15% POE-(20)-S-trioleate and 85% M812.



4.1.3. Emulsification of M812/T85/S85 mixtures

4.1.3.1 M812/T85 mixtures

Table 4.1 lists data describing the rate of emulsification of M812/T85 mixtures in distilled water at 25°C. These results are typical of the information which was generated using the nephelometer during self-emulsification. The value of Rel I_{100} varied little between replicate experiments as can be seen from the values of the standard deviations. Values of Rel I_{100} above 50 units were typical of emulsions of good quality. At T85 contents of less than 15%^{w}/w emulsification was poor and values of Rel I_{100} fell below 5 units. Similarly low values of Rel I_{100} were typical of mixtures with T85 contents greater than 60%^{w}/w. Essentially, mixtures with T85 contents outside these lower and upper limits did not 'self-emulsify'. For such mixtures the measurement of emulsification rate parameters was inappropriate. Within the range of more efficiently emulsifying mixtures (20-60%^{w}/w T85) the values of emulsification time ($t_{I\%}$) showed distinct trends. The region 30-45%^{w}/w T85 contained mixtures with the lowest $t_{I\%}$ values with a minimum emulsification time at 40% T85. Either side of this region $t_{I\%}$ values rose steeply, particularly at high T85 contents (Figure 4.17.). Variation in emulsification times between replicate experiments became consistent provided that at least five replicate experiments were undertaken. The standard deviations were between five and ten percent of the mean value unless emulsification was very poor.

Table 4.1. Rate of emulsification of M812/T85 mixtures in distilled water at 25°C (mean and standard deviation of five replicates)

MIXTURE COMPOSITION %w/w			$t_{1\%}$ (seconds)				D_{0-50}
M812	T85	REL I_{100}	t_0	t_{50}	t_{75}	t_{90}	(sec.$\%^{-1}$)
80	20	80.0	4.8	9.1	12.8	19.1	0.087
		3.3	0.4	0.9	2.7	5.2	0.011
75	25	80.6	3.6	8.3	11.2	15.5	0.093
		2.3	0.4	0.7	1.0	1.8	0.013
70	30	81.7	3.2	6.5	8.5	11.3	0.065
		3.7	0.3	0.4	0.8	1.4	0.009
65	35	71.8	3.0	6.3	8.2	10.7	0.067
		1.9	0.2	0.4	0.5	0.9	0.006
60	40	56.2	2.7	5.8	7.5	9.1	0.062
		0.7	0.3	0.4	0.4	0.4	0.007
55	45	36.8	2.1	5.7	8.0	12.2	0.073
		1.7	0.2	0.4	0.5	2.4	0.004
50	50	20.4	2.4	8.7	16.4	32.9	0.126
		1.4	0.7	1.5	4.9	10.7	0.024
40	60	11.7	3.0	11.1	20.5	36.5	0.163
		0.8	0.5	1.4	2.5	5.0	0.031

Figure 4.17. Emulsification times ($t_{1\%}$) of M812/T85 mixtures in distilled water at 25°C.

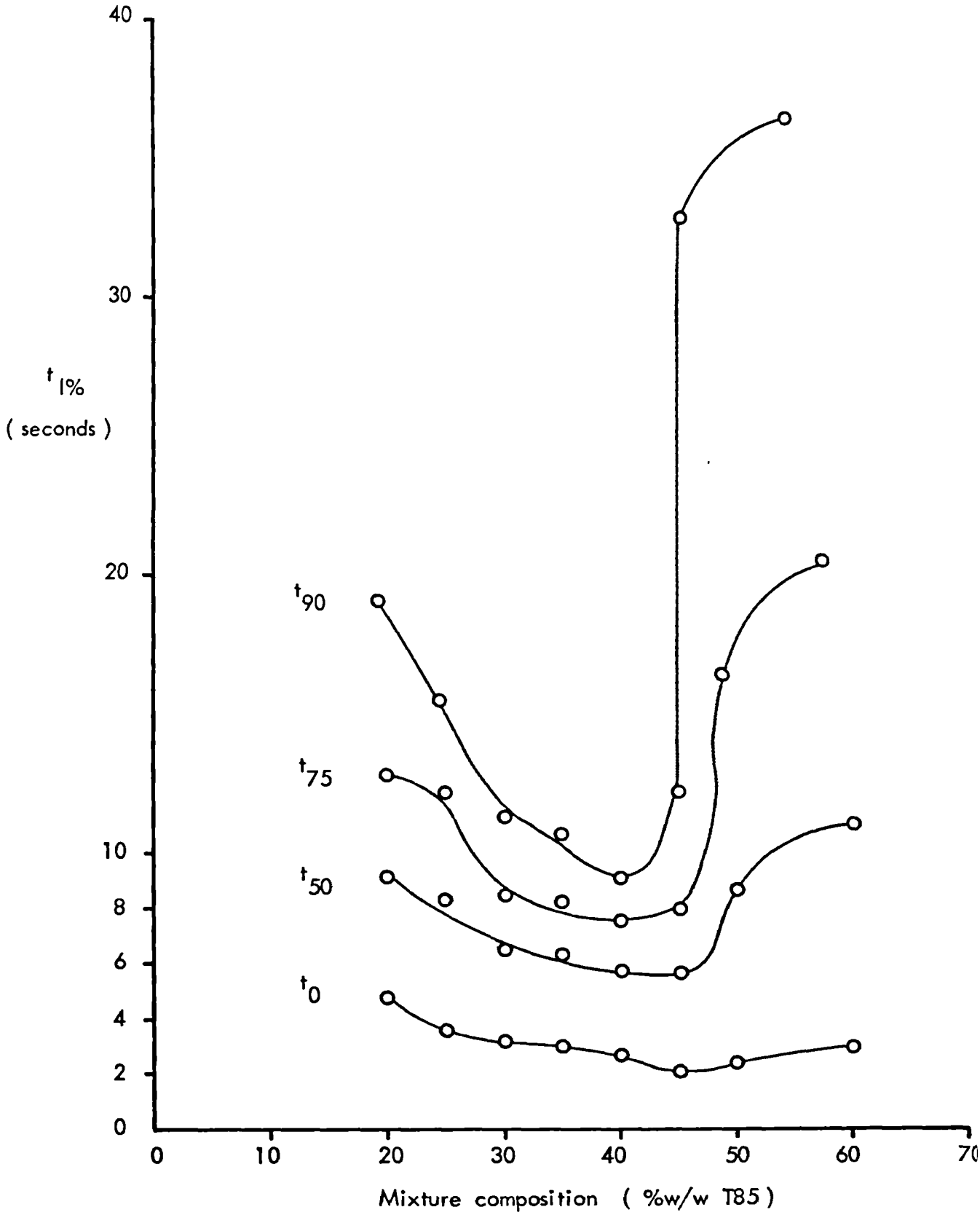


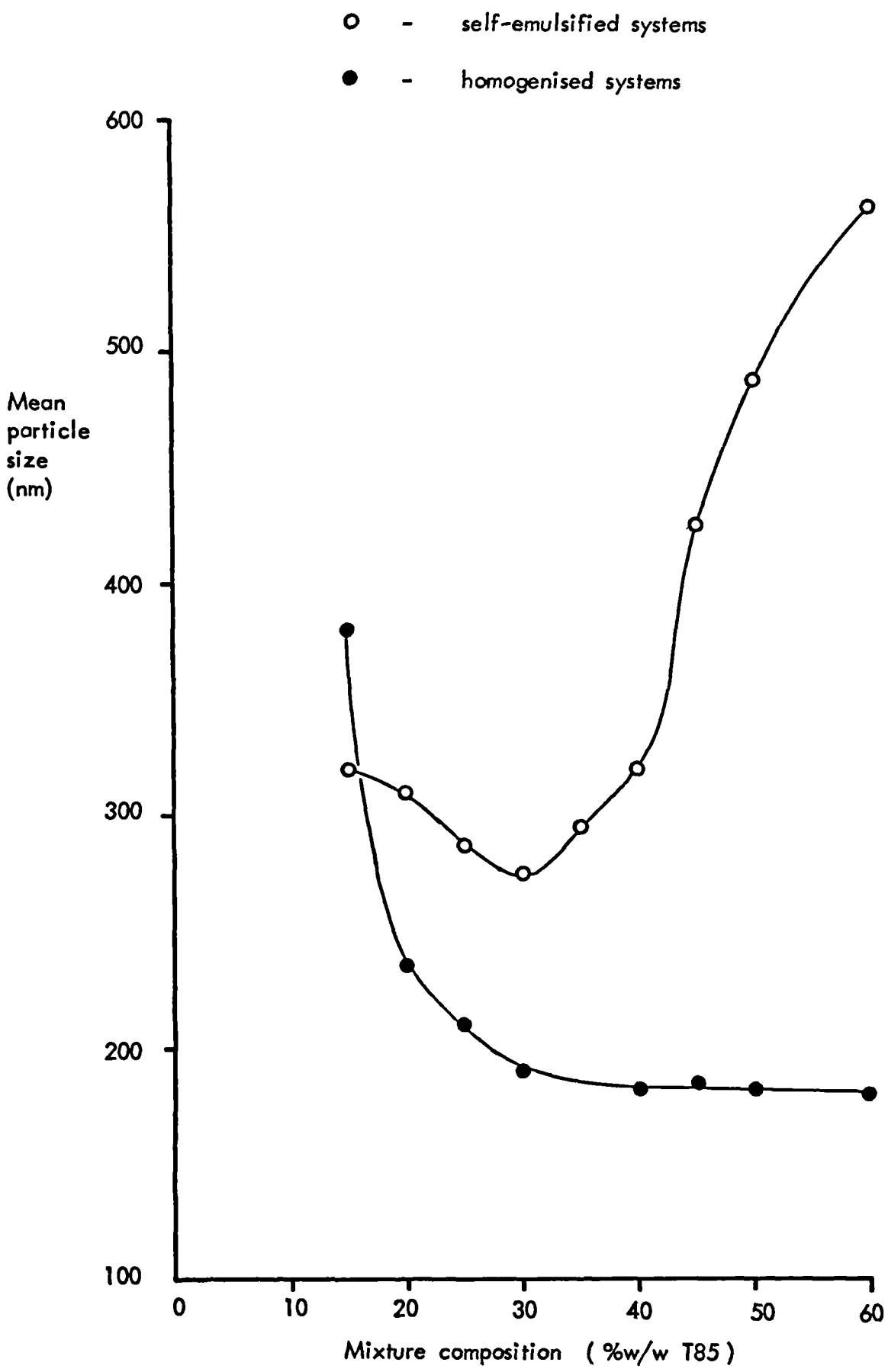
Figure 4.17. shows that generally values of $t_{50\%}$, $t_{75\%}$ and $t_{90\%}$ changed in the same manner. This illustrates the point that a change in emulsification rate was reflected in the whole curve of $I_{\%}$ against time; the general shape of each curve remaining similar. For this reason it is possible to use a single parameter to describe emulsification time. In discussion of other results the parameter $t_{50\%}$ will often be used as an indication of emulsification rate.

An illustration of the information available from the Coulter Nanosizer is given by Table 4.2. Self-emulsified systems of M812/T85 mixtures had various mean particle sizes; a minimum occuring in the region of 20-40%^{w}/$_{w}$T85 (Figure 4.18.), the inflexion being at 30%^{w}/$_{w}$T85. At higher T85 concentrations the emulsions formed by self-emulsification were of increasing mean size. The poorer quality of these emulsions was also indicated by higher standard deviations of the mean size and by substantially higher values of the polydisperisty index (a measure of the size range, see section 3.2.5.2.). At T85 concentrations of less than 2o%^{w}/w the measurements made using the Nanosizer were less appropriate. The measurements made of emulsions formed using the 85%M812/15%T85 mixture illustrate this point. For this system there was a sharp increase in standard deviation and polydispersity index. Measurements made of emulsions formed by mixtures containing less than 15%T85 were quite misleading. The particle size appeared to drop to

Table 4.2. Assessment of quality of self-emulsified and homogenised emulsions of M812/T85 mixtures in distilled water at 25°C.

MIXTURE COMPOSITION %w/w		SELF-EMULSIFIED SYSTEMS			HOMOGENISED SYSTEMS		
		PARTICLE SIZE (nm)		P.I.	PARTICLE SIZE (nm)		P.I.
M812	T85	Mean	S.D.		Mean	S.D.	
85	15	320	22	7	380	18	4
80	20	310	3	2	235	6	3
75	25	287	3	2	210	4	3
70	30	278	2	2	189	2	1
65	35	295	2	4	176	6	3
60	40	321	12	5	182	6	2
55	45	423	25	7	186	4	3
50	50	487	24	8	182	7	3
40	60	562	33	9	180	5	4
-	100	-	-	-	135	1	4

Figure 4.18. Mean particle sizes of self-emulsified and homogenised emulsions of M812/T85 mixtures in distilled water at 25°C.



approximately 250nm. However, microscopical examination of the emulsions showed that large particles and some free oil were present. Therefore the size range for these emulsions was too wide to allow realistic assessment using the Nanosizer. It is likely that the machine measured the mean size of some fine particles present which did not represent the mean size of the total emulsion. Indeed the dispersions may have been bimodal, judging by their appearance under the microscope. In summary, the Coulter Nanosizer must be used with caution for measurement of polydisperse systems and should be accompanied by microscopical examination or another sizing method which would detect the presence of large particles.

Returning to the effect of mixture composition on emulsification properties of M812/T85 mixtures, it would seem that, generally, fine emulsions with narrow size ranges were formed at rapid rates. The curves plotting emulsification time and particle size of resultant emulsions had similar shapes. Although the mininum value of the particle size occurred at a slightly lower T85 content than the corresponding minimum of emulsification time, there is a distinct range in which the two curves overlap. The mixture 70%M812/30%T85 was regarded as the model 'self-emulsifying' mixture and is used as a standard in further studies.

The particle sizes of homogenised emulsions (of the same concentration) are also listed in Table 4.2. The measurements of emulsions formed from the 15%^{w}/wT85 mixture illustrate poor emulsion quality. Even after homogenisation the particle size was high and variable. As T85 concentration increased the homogenised systems became fine and above 40%^{w}/wT85 had mean sizes of 180-190nm. Therefore whilst mixtures above 50%T85 were poorly self-emulsifying, fine emulsions were produced when large amounts of energy were available for emulsification (Figure 4.18).

In order to compare the efficiencies of self-emulsification M812/T85 mixtures may be grouped using the following categories.

	Typical %^{w}/wT85 at 25^{o}C in distilled water	General characteristics
Type A	5-15%	Mixtures dispersed at various rates to form poor emulsions with extremely wide size ranges.
Type B	20-40%	Rapid self-emulsification resulted in fine, evenly dispersed particles.
Type C	45-60%	Mixtures dispersed slowly forming coarse emulsions with wider size ranges than emulsions formed by Type B mixtures.
Type D	70-100%	Little emulsification occured unless large amounts of energy were available.

Plates 1 to 3 are photomicrographs of typical emulsions formed by Type A, B and C mixtures. Most of the particles formed by Type B mixtures were too fine to be resolved by light microscopy whereas emulsions formed by Type A and Type C mixtures included some large particles. In the case of Type A mixtures the large particles were substantially oil. Type C mixtures included large particles of undispersed oil/surfactant material.

The above four classes will be used to describe broad differences between oil/surfactant mixtures in future discussion.

During a study of emulsion stability Lee has considered self-emulsification of xylene/surfactant mixtures with regard to formulation of herbicidal emulsifiable concentrates[38]. As surfactant concentration was increased subjective assessments of the 'spontaneity' were made using the CIPAC[41] method. 1%^{w}/w surfactant was required for self-emulsification and efficiency improvedup to surfactant concentrations of 5% after which droplet size increased. Above 25% surfactant emulsification was poor until the surfactant concentration was high enough (60%) to form a slowly dispersing solubilized system. Similar behaviour has been observed by other workers[170-171]. The deterioration in spontaneity as surfactant concentration is increased in such mixtures is generally thought to be due to increasing viscosity. Higher viscosity would hinder

Plates 1-3 Appearance of typical emulsions formed by Type A, B and C mixtures in distilled water at 25°C.

100μm

Plate 1. Type A

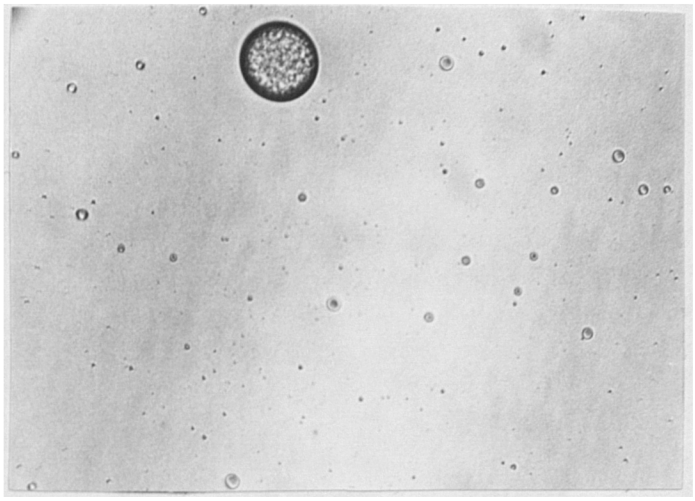


Plate 2. Type B

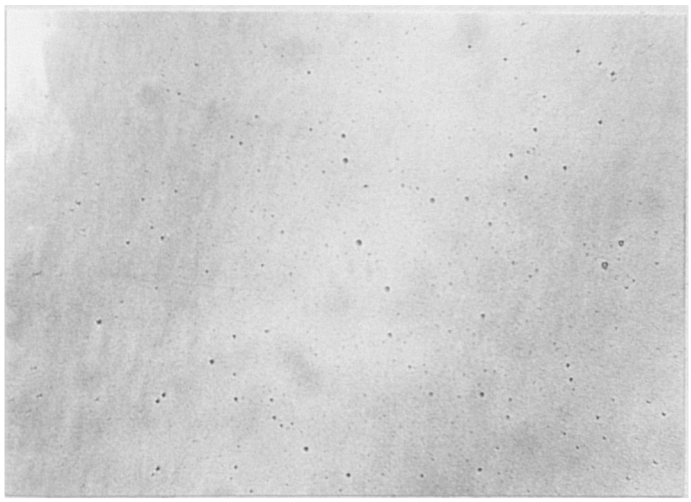
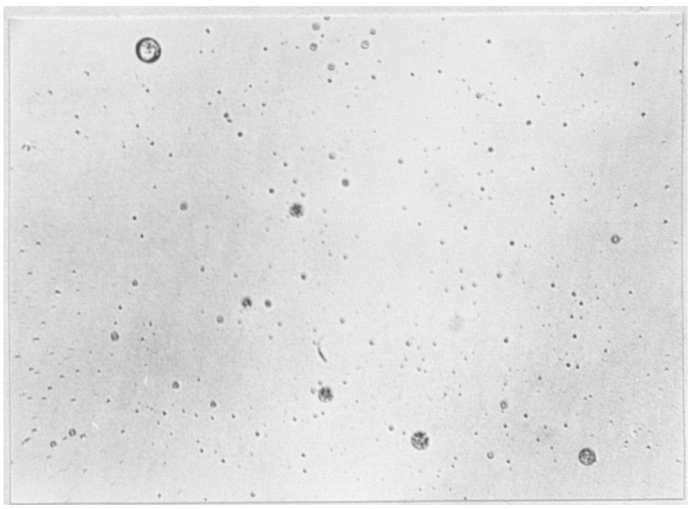


Plate 3. Type C



the diffusion of surfactant molecules up to and across the interface and hinder penetration of water molecules in accordance with the Stokes-Einstein equation:

$$D = KT/6\pi .a.\eta \qquad (4.1)$$

In addition, high viscosity would dampen interfacial turbulence and therefore hinder mechanical rupture of droplets. The increase in spontaneity between 1% and the optimum 5% in Lee's work[38] was explained by a progressive lowering of interfacial tension.

Initially, it would seem that M812/T85 mixtures exhibited similar properties to the xylene/surfactant mixtures examined by Lee. However, a closer look at the two sets of materials reveals significant differences. Firstly the viscosities of the M812/T85 mixtures were well over ten times higher than corresponding xylene/surfactant mixtures. Infact the optimum xylene/surfactant mixture of 5% surfactant had a viscosity below 1mPa.s. Spontaneity deteriorated as surfactant content rose to 20% when the viscosity was about 2mPa.s. In comparison, the optimum self-emulsification of M812/T85 mixtures (at a concentration of 30% surfactant) occurred when mixture viscosity was 39mPa.s.

The densities of the oil/surfactant mixtures may be important in certain circumstances[57,61,171]. The reason is generally considered to be that the differences between velocities of the continuous and dispersed phase lead to

differences in shearing strees. Thus large differences in density between the continuous and dispersed phase ($\rho_c - \rho_d$) favour droplet deformation and rupture[50]. The values of ($\rho_c - \rho_d$) for the xylene/surfactant systems studied by Lee[38] were approximately three times greater than those for the M812/T85 systems examined in this study. However despite the unfavourable viscosities and densities of the M812/T85 mixtures they resulted in finer emulsions with narrower size ranges than the 5% surfactant in xylene mixtures.These observations suggest that the effects of viscosity and density on hydrodynamic instability were of little significance to emulsification of M812/T85 mixtures.

Previous workers, e.g. Mustafa[172], have assumed that interactions between materials at an interface are related to the phase behaviour of bulk materials, which can be studied comparatively easily. It is possible to envisage how oil/surfactant mixtures may mix with an excess of water in relation to the concentration of surfactant present in the oil/surfactant mixtures. As a result of Mustafa's work[172] it has been proposed[42,97] that formation of liquid crystalline material, at the interface of an oil/surfactant mixture and excess water, may aid dispersion. The mechanism was explained by considering the interfacial pressures generated by formation of liquid crystal. If interfacial pressures

were high they may result in spontaneous expansion of the interface causing formation of small droplets.

Examination of the phase behaviour of Lee's[38] xylene/surfactant/water systems suggests that his 5% surfactant mixtures would not have formed liquid crystalline phases when mixed with excess water. Infact liquid crystals were not formed until the surfactant concentration was greater than 35%. The latter were poorly emulsifying mixtures. Lee considered that formation of gel-like liquid crystalline layers would retard further penetration of water and hence reduce the rate of disintegration of the bulk oil/surfactant mixture. One might assume that the spontaneous emulsification of Lee's mixture of 5% surfactant in xylene can be explained by strong adsorption of surfactant at the oil/water interface causing extremely low interfacial tension. Spontaneous emulsification under these conditions can be explained thermodynamically by the relationship

$$\Delta G \text{ form} = \gamma . \Delta A - T . \Delta S_{disp.} \qquad (4.2.)$$

as explained in Chapter Two.

Superficially, the results of Lee and Mustafa appear to be contradictory. However Mustafa's study does not include the quality of resultant emulsions; it is based on a measure of 'spontaneity time' alone (this can be misleading as demonstrated by the M812/T85 system).

Furthermore Mustafa did not examine the phase behaviour of his systems at low water content, but only from 30% water upwards. Therefore liquid crystalline phases may not have been formed as soon as the oil/water surfaces met.

Examination of the phase behaviour of M812/T85/water systems reveals that certain properties of phase behaviour appear to favour spontaneous emulsification. The emphasis of the current discussion is on phases formed at low water content i.e. those interactions which are likely to take place as water initially penetrated an oil/surfactant mixture at 25°C.

Efficient emulsifying systems (Type B mixtures) were formed between 20 and 40%^{w}/wT85. With reference to figures 4.13. to 4.15. it can be seen that these mixtures were close to but below the region of high water solubilization. As explained in Chapter Two the interfacial tension in this region is low and approaches zero at the region of the phase inversion[109,115,187]. The low values of interfacial tension may be enough to explain the ease of emulsification of these mixtures. Another interesting feature of Figures 4.14 and 4.15 was the presence of the liquid crystalline phase (I_{LC3}). It is thought that this phase was a reversed hexagonal phase formed on addition of water to saturated micellar solution. It is conceivable that this liquid crystalline phase could have formed at the oil/water interface when

an oil/surfactant mixture was added to excess water. The liquid crystalline phase could have provided a direct path for continued water penetration causing rapid explosion of the interphase to form fine droplets. Similar suggestions have been noted by Van der Waarden[173]. The possibility of such a mechanism for self-emulsification is discussed further in section 4.1.3.3.

As the ratio of M812 to T85 was increased the size of the critical region of water solubilization became smaller and its critical temperature (the CST) became lower (Figs. 4.12 to 4.16). At a ratio of M812 80%/T85 20%^{w}/w the critical solubilization temperature was just above 25°C. If the T85 content was further reduced the CST dropped below 25°C. Figure 4.16 also shows that if water was gradually added to 15%T85 mixtures at 25°C the phase changes were as follows. When the micellar region (I_o) was saturated with water further addition of water caused phase separation into two distinct phases; one rich in surfactant, the other in oil. If more water was added to this mixture the surfactant-rich phase became birefringent, indicating the presence of liquid crystalline material, whilst the oil-rich phase remained unchanged. It is significant that these changes in phase behaviour corresponded to the transition between Type B and Type A mixtures. The wide size range of emulsions formed by Type A mixtures may be explained by the initial separation

of an oil-rich phase (which was poorly emulsified) and subsequent dispersion of the surfactant-rich phase forming droplets. As the surfactant concentration was reduced below 15% in the original I_o mixtures the volume fraction of the separating oil-rich phase became larger and corresponding emulsions contained increased proportions of poorly dispersed oil.

The progressive decline in efficiency of self-emulsification as T85 concentration in the original I_o mixtures was increased (Type C and Type D mixtures) seemed to correspond to increased formation of gel-like lamellar liquid crystal. Figures 4.8 to 4.11 show that, at 25°C, the addition of water to I_o phases containing over 50%^{w}/wT85 resulted in the formation of two phase systems (II_{o+LC}).
As M812 concentration in the original mixture decreased the addition of water resulted in more viscous liquid crystalline phases. Mixtures containing less than 30%M812 (Type D mixtures) on addition of water produced semi-solid viscoelastic mixtures.

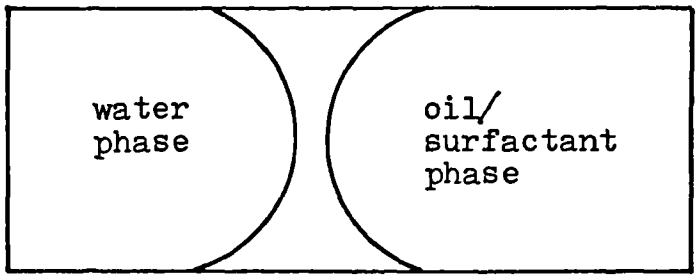
Typically Type C mixtures appeared to be 'partially dispersed'. In some cases water had penetrated the bulk only to become stranded, forming typical large w/o/w particles. But the change from Type C to Type D mixtures was gradual, in agreement with Lee's work[38].
It would seem that formation of viscoelastic lamellar phases hindered emulsification. In addition, one would expect that interfacial tension increased with T85

concentration as the critical solubilization temperature (or PIT) became higher.

The solubilized systems formed by Lee's 60% surfactant in xylene mixtures were probably formed spontaneously due to molecular diffusion of surfactant which formed micelles in the excess aqueous phase. Such systems were not formed by M812/T85 mixtures because T85 did not form aqueous micelles.

Visually, emulsification of Type B mixtures appeared to take place by erosion of very fine particles from the oil/water interface. During this process the bulk oil/surfactant mixture remained a discreet mass (unless mechanically sheared) which reduced in size as erosion proceeded. Differences in the appearance of interfacial disruption for a range of M812/T85 mixtures could be viewed by light microscopy as described in section 3.2.8. Plates 4-8 are photomicrographs (all to the same scale) of interfacial disruption a few seconds after M812/T85 mixtures met a distilled water surface at 25°C. In all cases replicate experiments of the interaction of each mixture with water produced characteristic textures.

The surfaces met along a line running vertically across each photograph, beginning with the waterphase on the left and the oil/surfactant phase on the right of each photograph, as shown below.



When the two surfaces met in Plates 4-7 the water phase typically penetrated the oil/surfactant (I_o) phase releasing streams of oily particles into the water phase. Water droplets did not appear in the oil phase. Thus the bulk oil phase remained clear (to the right of each photograph). The textures formed by the streaming of oil particles into the water phase were indicative of the particle size of droplets resulting from interfacial disruption.

Plate 4 shows the emulsification of the 85%M812/15%T85 mixtures. Large droplets and elongated slicks of oil streamed into the water phase. The particles formed by the 20% T85 mixture (plate 5) were considerably finer. These two photographs show the transition between Type A and Type B behaviour. M812/T85 mixtures containing less than 15% T85 were very poorly dispersed. Plate 6 shows emulsification of the 30% T85 mixture. The high efficiency of emulsification is indicated by the fine, even texture produced close to the interfacial region. Emulsification of the 50% T85 mixture (Plate 7) was also even but was coarser than that of the 30% T85 mixture. Such behaviour was indicative of the transition to Type C

Plates 4-7. Interfacial disruption caused by penetration of distilled water into M812/T85 mixtures at 25°C.

200μm

Plate 4. 85%M812/15%T85 mixture

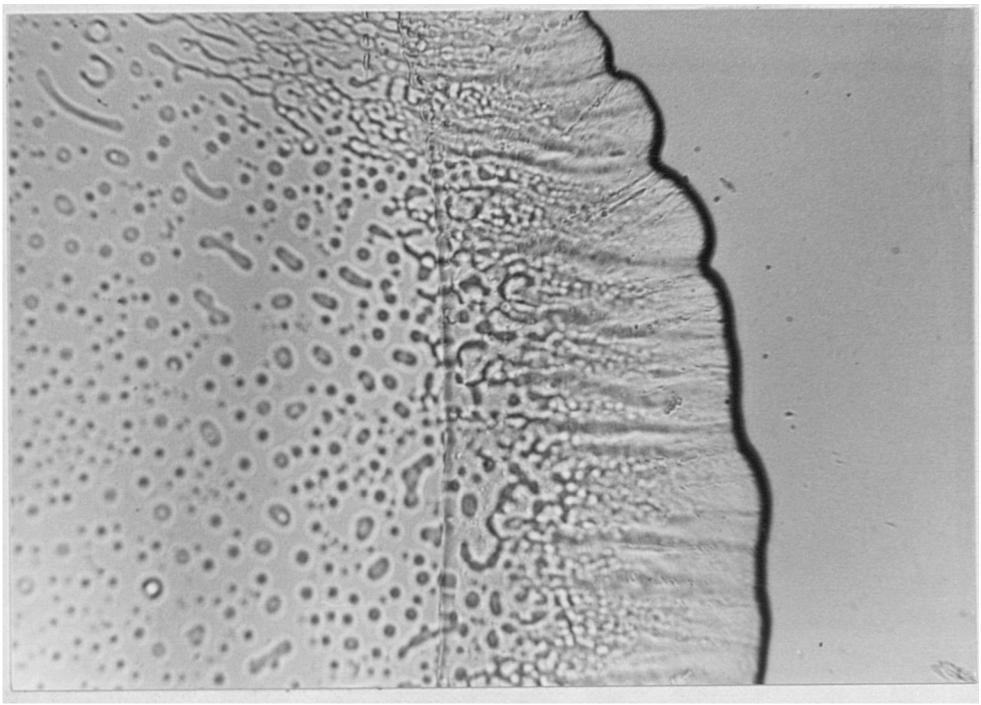
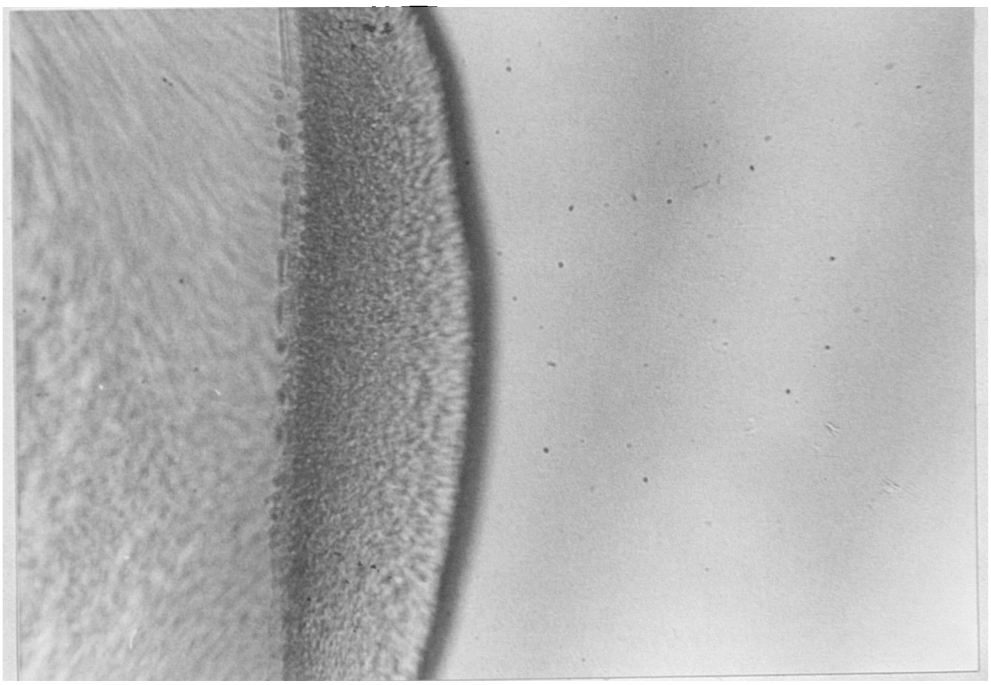


Plate 5. 80%M812/20%T85 mixture

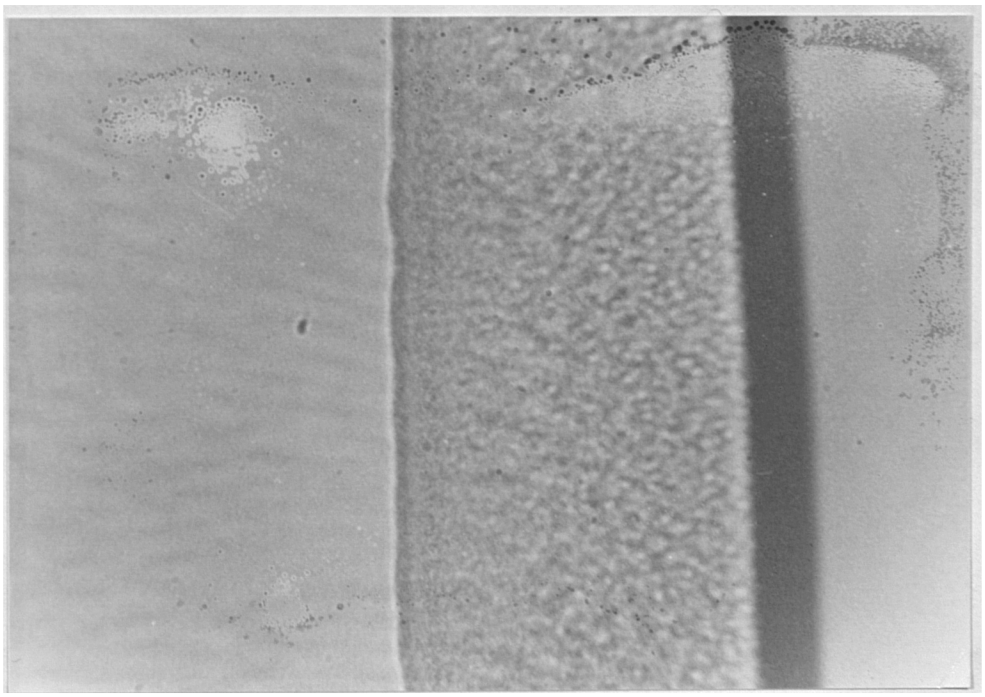


Plates 4-7. Interfacial disruption caused by penetration of distilled water into M812/T85 mixtures at 25°C.

200μm

Plate 6. 70%M812/30%T85 mixture

Plate 7. 50%M812/50%T85 mixture

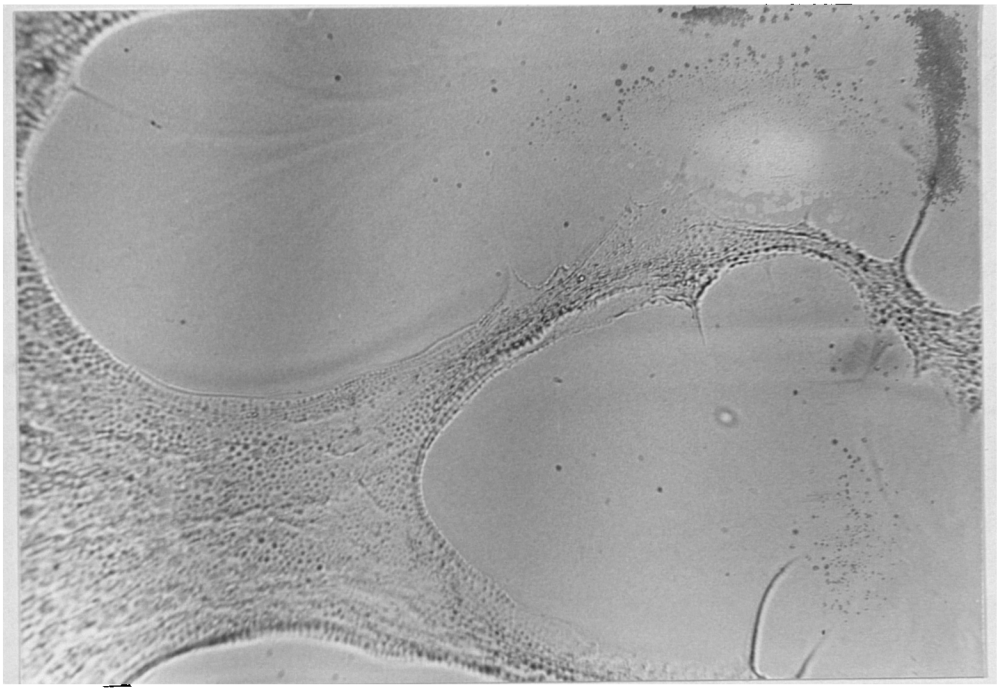


behaviour. Plate 8 shows the interaction of 20% M812/80% T85 (a typical Type D mixture) with water. In this case oily particles were not dispersed into the water phase. Instead the water phase was gradually dissolved into the oil/surfactant phase. Gel-like liquid crystalline phases were formed during this process and could be detected at the interface by polarizing microscopy. Hence Type D mixtures failed to self-emulsify. Mixtures containing between 50% and 80% T85 (which represent the transition between Type C and Type D character) behaved as intermediates between the two extremes of behaviour shown in Plates 7 and 8.

Subsequent to the initial interfacial disruption emulsification ceased due to the lack of water available for further penetration. The interfacial region could then be observed in a static state. This technique highlighted the differences in texture of emulsions formed by Type B oil/surfactant mixtures. Plates 9, 10 and 11 show the interfacial regions formed by water and 20%, 30% and 50% T85 in M812 mixtures. The 20% T85 mixture (Plate 9) was unevenly dispersed in comparison with the 30% T85 mixture (Plate 10). The 50% T85 mixture (Plate 11), whilst even, had a coarse texture.

Plate 8. Interaction between the 20%M812/80%T85 mixture and distilled water at 25°C.

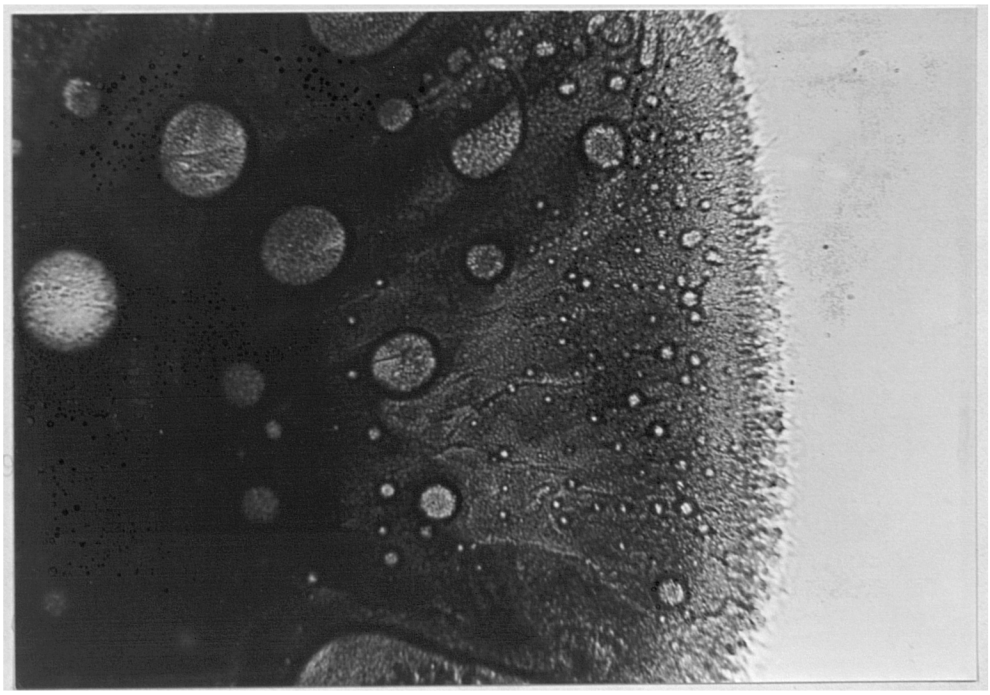
200μm



Plates 9-11. Appearance of the oil/water interface after cessation of emulsification (M812/T85 mixtures in distilled water at 25°C)

Plate 9. 80%M812/20%T85 mixture.

200μm



200μm

Plate 10. 70%M812/30%T85 mixture

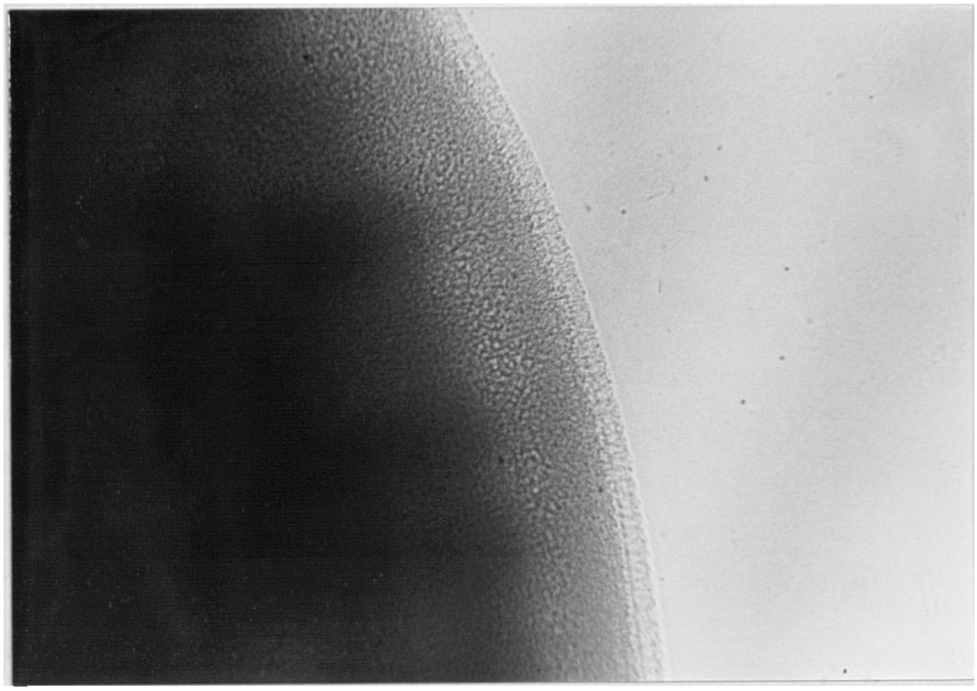
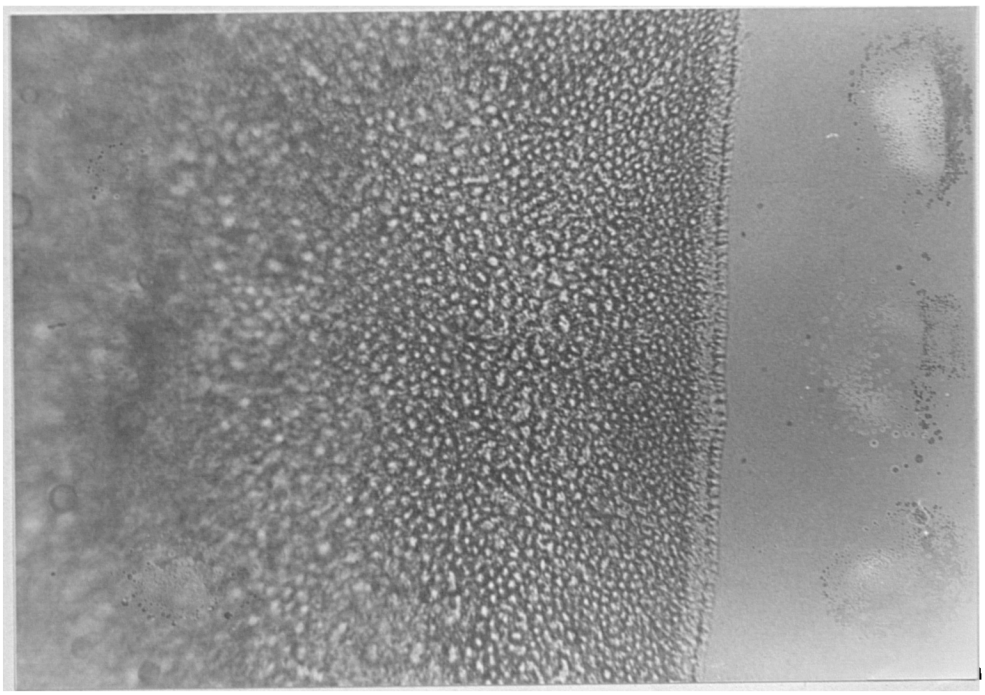


Plate 11. 50%M812/50%T85 mixture



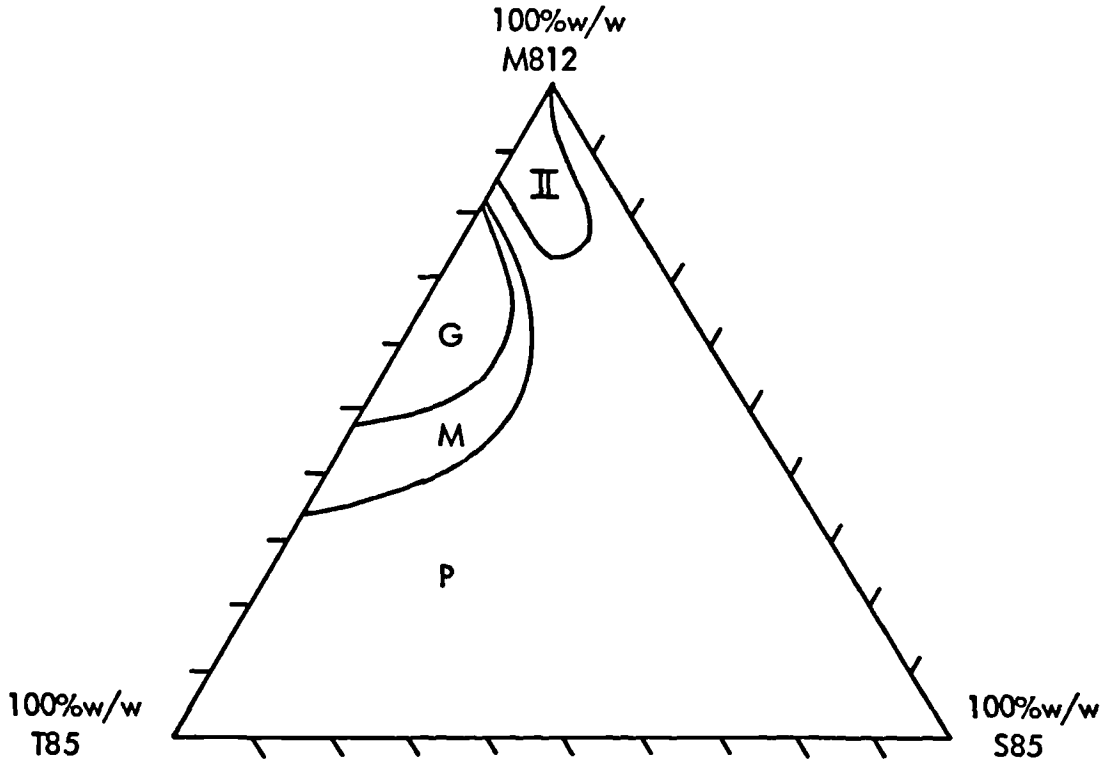
4.1.3.2. M812/T85/S85 mixtures

Surfactant pairs have been recommended for formulation of self-emulsifiable oil systems[38,42,97,98,174]. Most authors have agreed that a combination of hydrophilic and lipophilic surfactants result in more efficient self-emulsification than that of mixtures containing single surfactants. This view has developed speculatively as a result of observations of emulsion stability. It has long been known that mixed surfactants can produce more condensed interfacial films resulting in greater stability against coalescence[175-176]. Such complexes have been shown to reduce interfacial tension[177] and were most efficient when one surfactant was preferentially water soluble and the other was preferentially oil soluble[177-179]. Hence surfactants have been selected for self-emulsification along much the same lines as selection for emulsion stabilization; by the use of H.L.B. values (see section 2.4.).

The effect of inclusion of Span 85 (S85), the lipophilic counterpart of T85, is considered in this section. The behaviour of the more hydrophilic surfactant, T80, is considered in a later section.

Using the value Rel I_{100} as a guide it was possible to map out regions of good, moderate and poor self-emulsification for M812/T85/S85 mixtures (figure 4.19). Combinations of M812 with S85 alone were extremely poorly

Figure 4.19. Guide to the efficiency of self-emulsification of M812/T85/S85 mixtures in distilled water at 25°C.



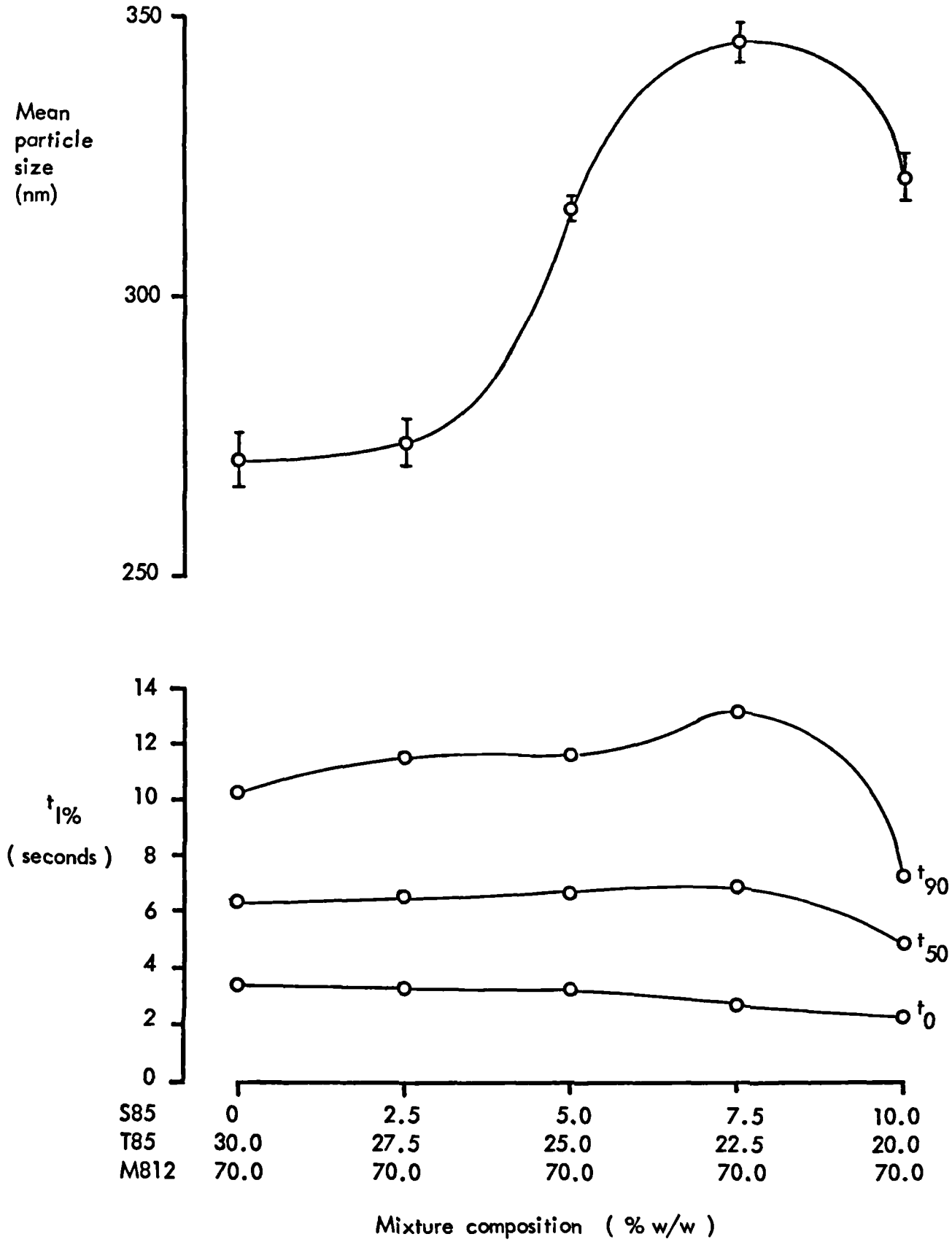
G - good : Rel $I_{100} > 25$

M - moderate : $25 >$ Rel $I_{100} > 10$

P - poor : Rel $I_{100} < 10$

emulsifying. Infact the range of total surfactant concentration which produced efficiently emulsifying mixtures became progressively narrower as the ratio of S85 to T85 was increased. A closer examination of the self-emulsification of M812/T85/S85 mixtures was conducted by comparison with the standard 70%M812/30%T85 mixture. Figure 4.20. illustrates the result of including S85 at the expense of T85; that is, keeping a constant concentration of surfactant by weight but increasing its lipophilic character. The values of emulsification time ($t_{I\%}$) did not change greatly for mixtures containing up to 7.5%S85 although after 7.5% there was an apparent fall in $t_{I\%}$. The value of t_0 dropped steadily which may have been a manifestation of the decreasing viscosity. The particle sizes of the emulsions were significantly different; a steep rise in mean size occured between 2.5 and 7.5% S85 content. An apparent fall in particle size occured between 7.5 and 10.0% S85. Microscopical examination of the emulsions showed that the number of large particles increased especially between 5 and 10% S85. This represented a transition between Type B and Type A mixtures. At S85 contents of 10% and above the emulsions formed were widely dispersed and of much poorer quality than those formed by mixtures containing 0-5% S85. Essentially, the inclusion of S85 at the expense of T85 had the same effect as increasing the oil content, resulting in Type A mixtures above 7.5% S85. This phenomenon is explained by a lowering of the CST to temperatures below 25°C when the concentration of S85 exceeded 7.5%^{w}/w. Plates 12-14 are micrographs of

Figure 4.20. Self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C.



Plates 12-14 Emulsions formed by self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C.

100μm

Plate 12 70%M812/25%T85/5%S85

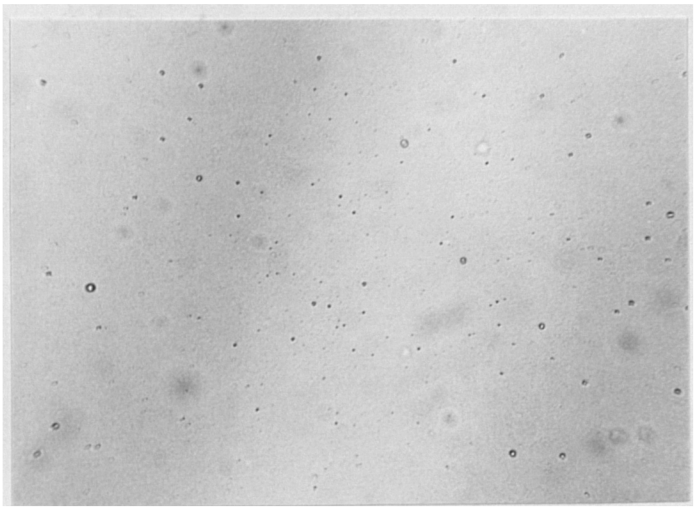


Plate 13 70%M812/22.5%T85/7.5%S85

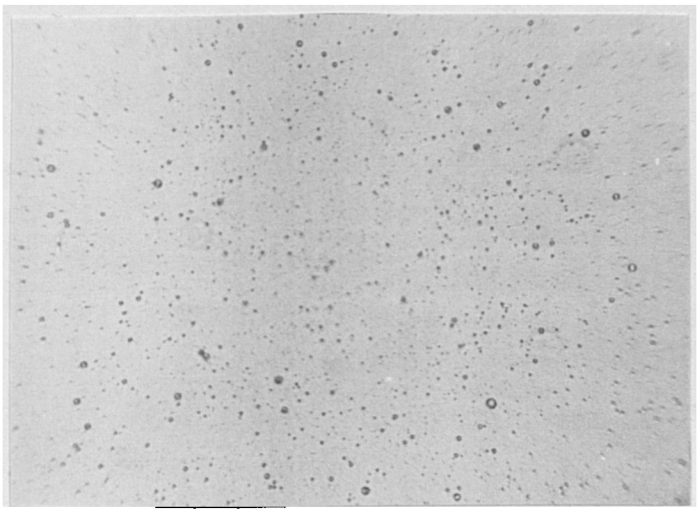
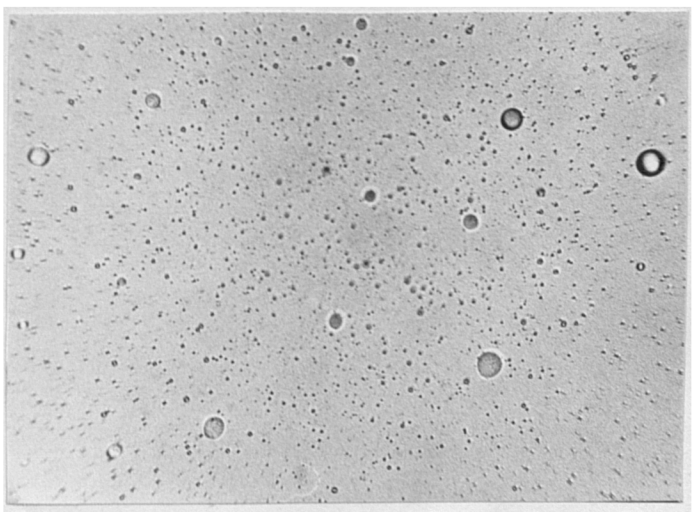


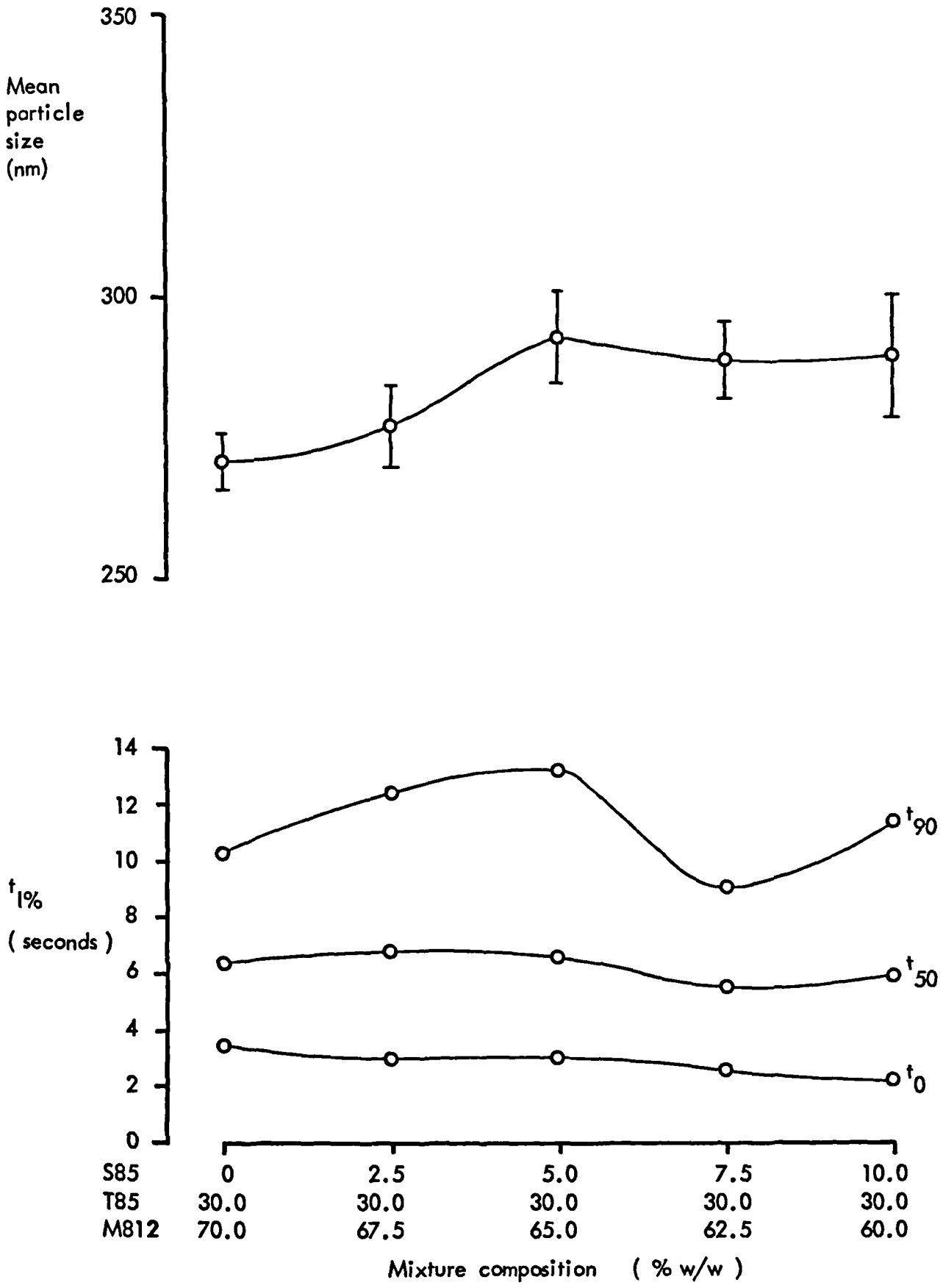
Plate 14 70%M812/15%T85/15%S85



emulsions formed from mixtures containing 5%, 7.5% and 15%^{w}/w S85 respectively in distilled water at 25°C. (The total surfactant concentration remained constant at 30%^{w}/W). Plates 12 and 13 show the deterioration in quality of emulsions formed by mixtures containing over 5%^{w}/w S85. The typical, large particles formed by a Type A mixture can be seen in Plate 14. The lowering of the CST (and PIT) caused by inclusion of lipophilic surfactants has been described by Shinoda[112].

S85 was also added to the model mixture at the expense of M812, keeping the T85 concentration constant at 30%^{w}/w. The properties of this series of mixtures were similar to those of the series previously described. Figure 4.21 shows that efficiency of self-emulsification deteriorated gradually as S85 concentration was increased to 5%, as indicated by particle size and $t_{90\%}$ measurements. Between 5% and 10% S85, again, there was a transition to polydisperse emulsions and measurements became misleading. This behaviour is indicative of the importance of the nature of the oil in oil/surfactant systems[121]. Infact the Nanosizer did indicate that emulsion quality continued to deteriorate between 5% and 10% S85 by displaying a rise in polydispersity index. Nevertheless the experiments represented by Figures 4.20 and 4.21 serve to illustrate the likelihood of obtaining misleading estimates of mean particle size when using the Nanosizer for sizing polydisperse systems.

Figure 4.21 Self-emulsification of some M812/T85/S85 mixtures in distilled water at 25°C.



However in practice the transition from Type B to Type A mixtures resulted in a large change in emulsion quality which could easily be detected by microscopy and often by visual inspection.

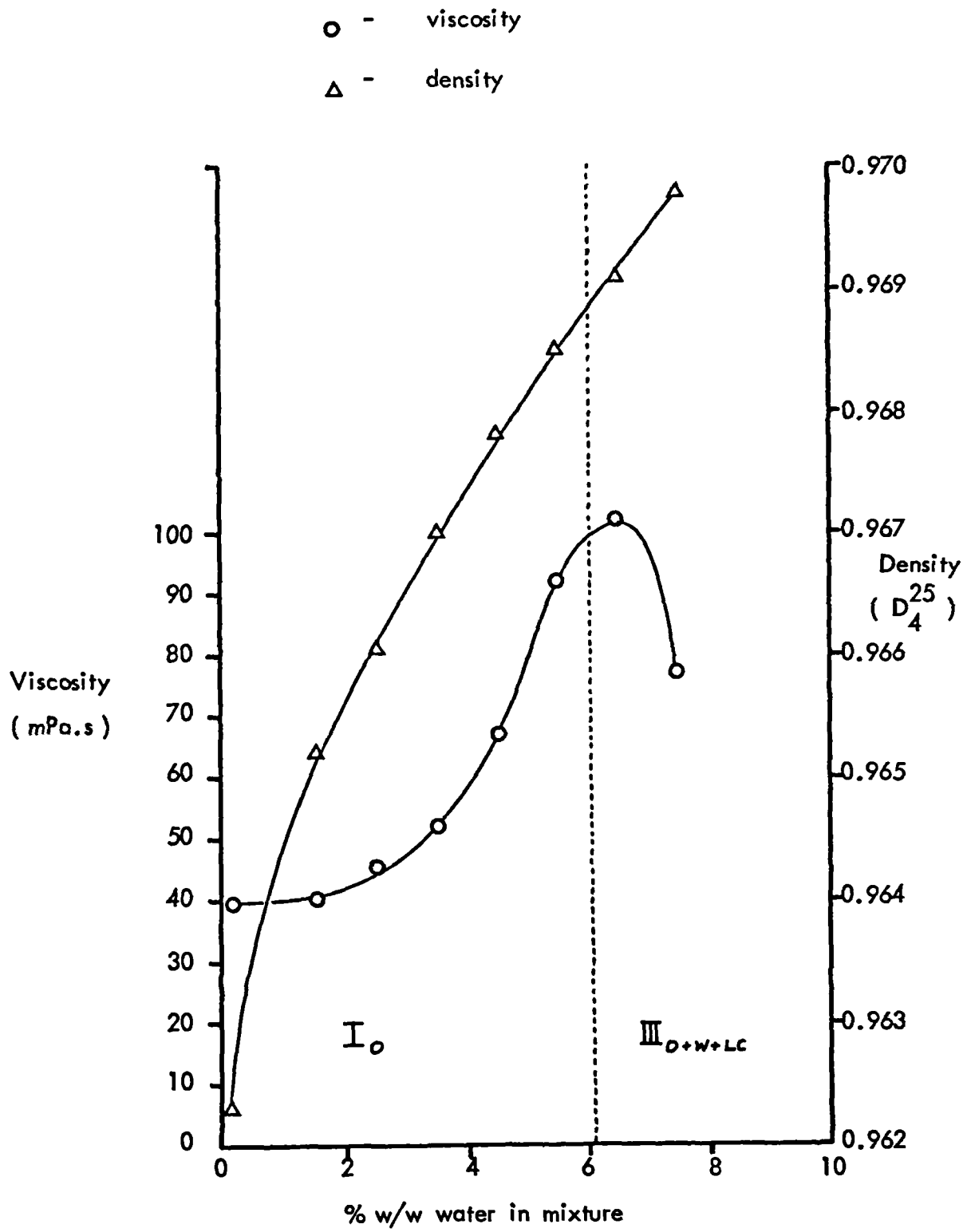
In summary, the effects of including S85 did not aid dispersion of the model M812/T85 mixture but instead modified the oil/surfactant/water interactions so as to move away from optimum conditions for dispersion. These changes were reflected in the phase diagram (Figure 4.7.) in which the liquid crystalline regions were reduced in size.

4.1.3.3. Effect of water content of original oil/surfactant mixtures on self-emulsification.

The effect of inclusion of water in the original mixture on self-emulsification of oil/surfactant mixtures was of interest for two main reasons. Firstly in relation to changes in moisture content of the raw materials, particularly T85. This has been shown to vary between approximately 4.8% and 5.1%^{w}/w (see section 4.1.1.). The viscosity of the I_o phase changed significantly as water was solubilized. Therefore it was important to determine whether self-emulsification was affected by increased moisture content. The second area of interest concerned the mechanism of self-emulsification. The process of emulsification might be regarded as a progressive dilution of the material in water. However it is unlikely that emulsification involves all the phase changes exhibited in an equilibrium phase diagram such as Figure 4.6. Therefore by studying the efficiency of self-emulsification at different compositions in the phase diagram one may discover which phases are most likely to self-emulsify.

Figure 4.22 illustrates the changes in density and viscosity as water was added to the model 70%M812/30%T85 mixture. The density increased steadily in a non-linear manner as water was added. The steepest increase in density seemed to occur at low concentrations of water suggesting that initial formation of micelles in the presence of small amounts of water produced profound changes in density of the bulk mixture. The subsequent increase in density

Figure 4.22 Effect of water content of mixtures on densities and viscosities at 25°C (70%M812/30%T85 plus water mixtures)



did not seem to be affected by the phase change to liquid crystal. In contrast the viscosity of the bulk mixture was not affected by the presence of small amounts of water but rose steeply as micelles became saturated with water. The steep rise in viscosity between 2% and 5% water probably reflected increasing packing order of micelles as their size increased. The phase transition to liquid crystal and subsequently the translucent III_{LC+o+w} mixtures resulted in a decrease in viscosity.

The efficiencies of self-emulsification of mixtures containing up to about 10%^{w}/w water are represented by Figure 4.23 (emulsification times) and Table 4.3. (emulsion quality). Emulsification time lengthened slightly as solubilized water in the I_o phase was increased. Increasing viscosity of the I_o phase probably explained the longer emulsification times although the effect was small in comparison with the increase in viscosity of the original oil/surfactant mixtures. The quality of emulsions formed by mixtures containing 0-5%^{w}/w H_2O, as expressed by the mean particle sizes and polydispersity indices, was unaffected by variation in water content. These results suggest that self-emulsification would not be affected by small changes in moisture content of raw materials as long as the I_o phase is maintained. There was a steep lengthening of emulsification time corresponding to the phase change to liquid crystal, when mixtures of higher water contents were examined. However resultant emulsions were of comparable quality to those

Figure 4.23 Effect of water concentration in original mixtures on emulsification time (70%M812/30%T85 plus water mixtures in distilled water at 25°C).

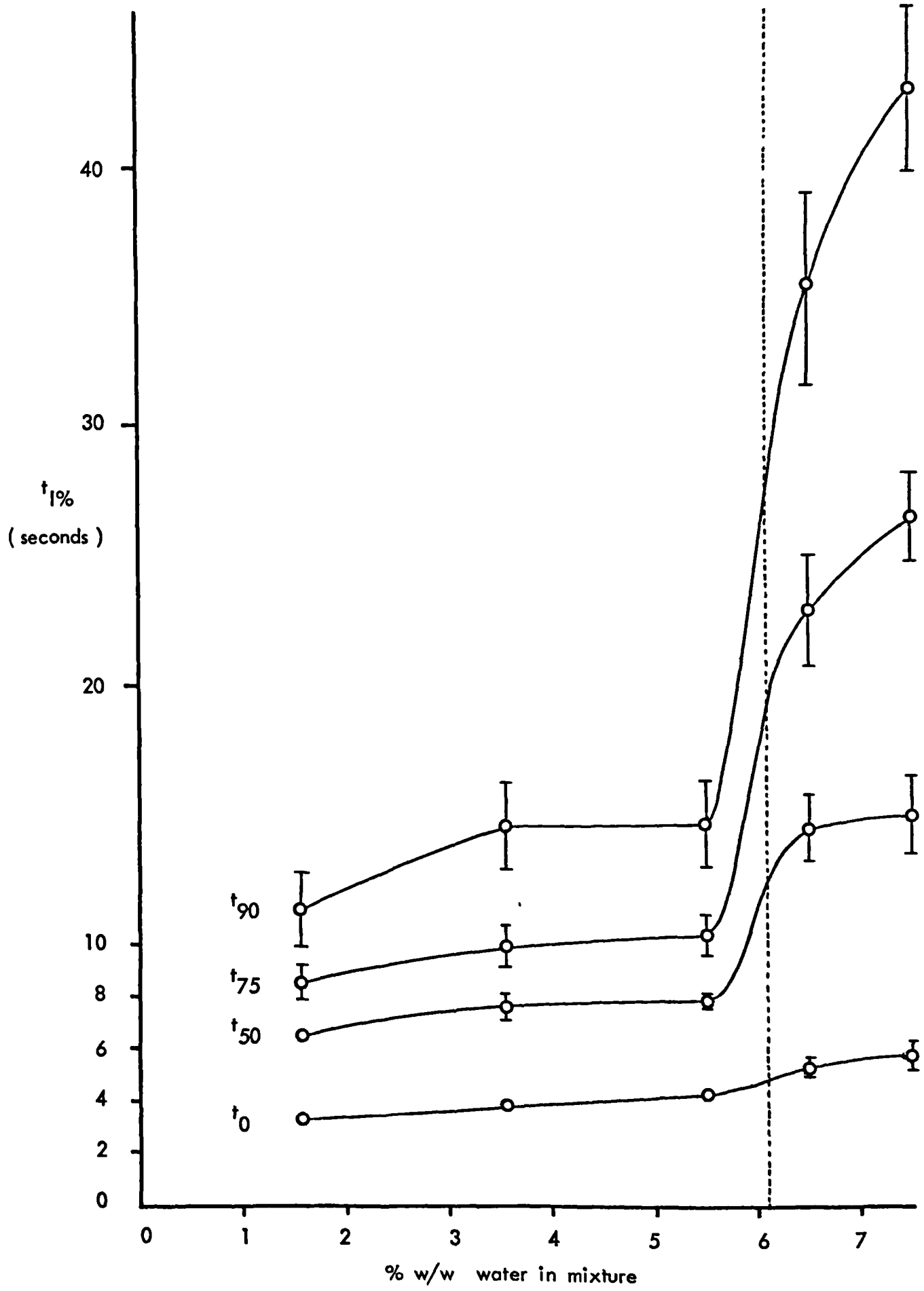


Table 4.3 Effect of water concentration in original mixtures on quality of emulsions formed by self-emulsification. (70%M812/30%T85 plus water mixtures in distilled water at 25°C)

Total water content of mixture %w/w	Phase characteristics of mixture at 25° C	Mean particle size after self-emulsification (nm)		P.I.
		Mean	S.D.	
1.57	clear, isotropic, I_o	265	4	2
2.55	I_o	267	4	1
3.54	I_o	266	6	1
4.52	I_o	268	5	1
5.51	I_o	270	6	1
6.49	I_{LC}	271	4	2
7.48	III_{LC+o+w}	279	4	2
8.95	III_{o+w+LC}	> 3000	-	-
11.41	III_{o+w+LC}	> 3000	-	-

formed by the I_o phase (see 6.49% H_2O, Table 4.3.). Further increase in water content resulted in formation of the three phase III_{o+w+LC} mixtures which did not self-emulsify. At 8.95% H_2O dispersion was very poor; after ten minutes agitation in the self-emulsification cell the dispersion was far too coarse to be sized using the Nanosizer.

The above results suggest that emulsification took place, most efficiently, from the I_o phase rather than by a progressive dilution process through other phases. However it is significant that the dispersion of the liquid crystalline phase did not result in emulsions of poorer quality (6.49% H_2O mixture). In view of this fact the mechanism for self-emulsification may have involved the formation of liquid crystal at the oil/water interface. When the oil/surfactant mixture was introduced to the water surface, solubilization of water within inverse micelles may have occured until the micelles were saturated. Subsequently cylindrical micelles (inverse hexagonal liquid crystal) may have formed locally at the interface, radiating towards the centre of the bulk oil/surfactant mixture (i.e. perpendicular to the oil-water interface). The cylindrical micelles may then have provided a route for further penetration of water through their hydrophilic cores causing large increases in surface pressure and disruption of the oil-water interface. By this means small emulsion droplets may have been expelled from the surface continually as penetration of water into the bulk mixture proceeded. Indeed, macroscopically, self-emulsification did appear to be

a process of erosion of small particles from the surface of the bulk mixture (see section 4.1.3.1.). In this context the longer emulsification time for dispersion of the pure liquid crystalline material may be explained by the fact that orientation of the cylinders in bulk hexagonal liquid crystal would be random. The random orientation may not have favoured such rapid penetration of water although finally resulted in dispersions of comparable quality.

4.1.3.4. Effect of Temperature on self-emulsification

An increase in temperature generally results in a decrease in the surface tension of a liquid. This phenomenon may be explained by considering that the attractive forces between surface molecules and the bulk liquid become less significant as the kinetic energies of surface molecules increase with temperature[180]. Similarly, for two immiscible liquids, interfacial tension decreases with increasing temperature. In addition the viscosities of liquids decrease with increasing temperature. These factors result in easier emulsification of one liquid in another as temperature is increased. Raising the temperature may also aid emulsification when surfactants are present[181], although such cases are more complex because interfacial adsorption is also affected by change in temperature. The effect of temperature on emulsification in the presence of non-ionic surfactants is particularly complex due to the sensitivity of the water-polyoxyethylene interaction.

Self-emulsification properties of the model mixture were examined at various temperatures. Control of temperature was difficult at high temperatures because the current self-emulsification cell was not 'water-jacketed'. However by measurement of temperature prior to and immediately after each experiment limits of $\pm$ 1°C were achieved. For routine work at various temperatures the self-emulsification cell would require modification to

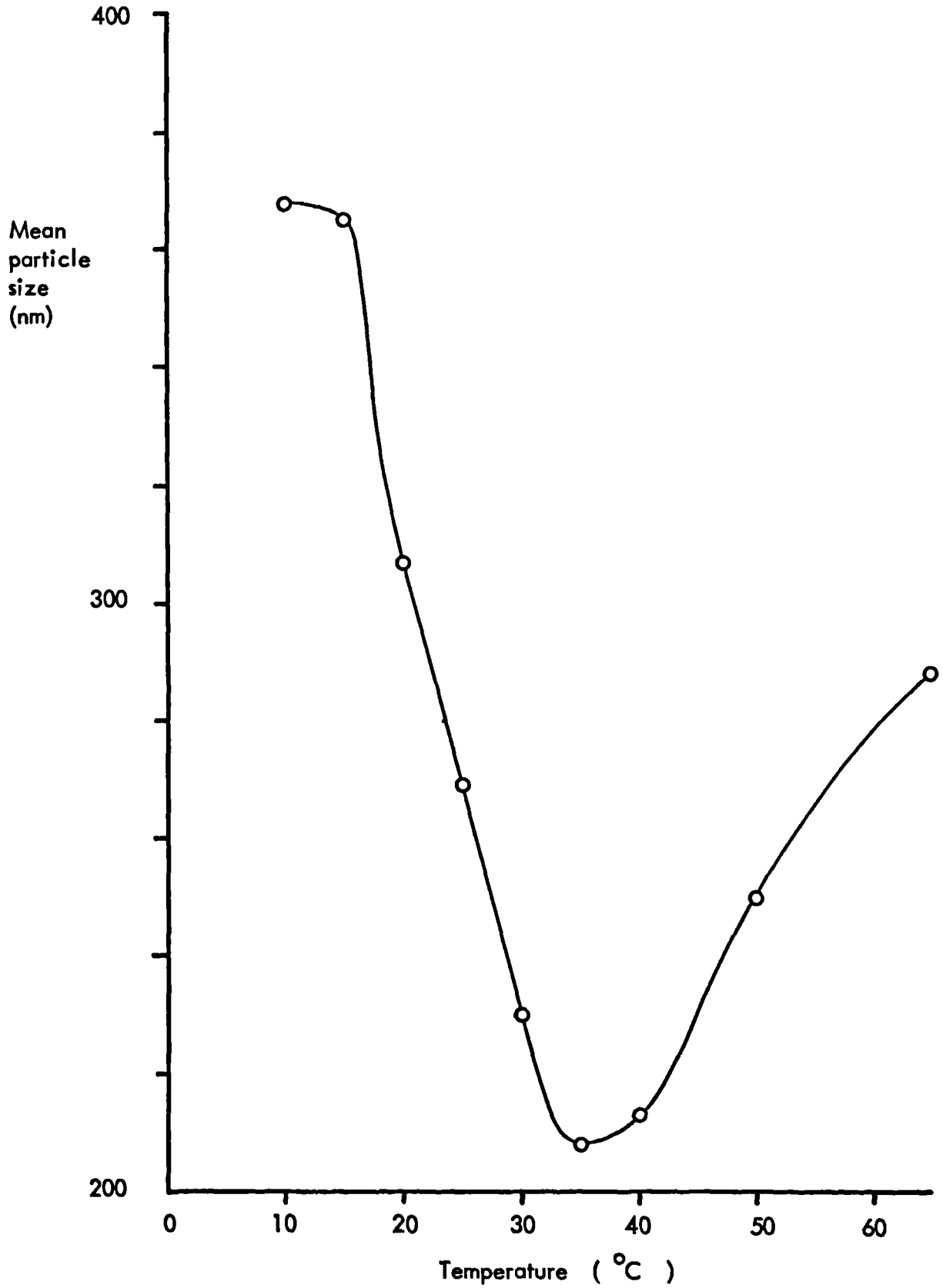
include a method of equilibrating the materials at fixed temperatures.

Parameters used to assess rate of emulsification (Table 4.4) did not change greatly over the range 9-38°C. Instead they seemed to fluctuate slightly within a narrow range (t_{50} values ranged from 6.1 to 7.1 seconds) without suggesting any particular trends which could have been related to temperature. However the values of Rel I_{100} changed significantly over the temperature range (Table 4.4.) suggesting that the particle sizes of the emulsions formed at different temperatures were profoundly different. Indeed this was the case. The mean particle size of resultant emulsions dropped steeply as the temperature increased from 10°C to 35°C (Fig. 4.24). Above 40°C the mean particle size began to rise. Above 50°C emulsions had still larger mean sizes and also wide size ranges (as expressed by the polydispersity index). At 50°C the polydispersity index was 1. At 60°C the value had risen to 3 and at 70°C the value was 5. Emulsions formed at 70°C or above were similar to those formed by Type A mixtures at 25°C. Infact over the range of temperature studied the emulsification behaviour of the 70%M812/30%T85 mixture transformed from Type C (<15°C) to Type B (20-50°C) and finally to Type A (>60°C). Such behaviour correlates with features of phase behaviour of the mixture and water at equilibrium. (Figure 4.14). During the studies of phase behaviour water was added gradually to the 70%M812/30%T85 mixture, allowing equilibration between

Table 4.4. Effect of temperature on rate of emulsification 70%M812/30%T85 in distilled water.

TEMP. (°C ± 1°C)	REL. I_{100}	$t_{1\%}$ (seconds) t_0	t_{50}	t_{75}	t_{90}	D_{0-50} ($sec.\%^{-1}$)
9	38.9 1.8	3.7 0.2	6.6 0.4	8.2 0.4	10.3 1.1	0.059 0.008
14	65.2 3.9	3.3 0.2	6.1 0.3	7.5 0.4	9.0 0.5	0.054 0.005
19	80.8 2.1	3.8 0.1	7.3 0.4	9.3 0.5	12.5 0.9	0.071 0.008
25	81.7 3.7	3.2 0.4	6.5 0.4	8.5 0.8	11.3 1.4	0.065 0.009
29	66.2 2.5	3.3 0.2	6.1 0.3	7.5 0.3	9.8 0.7	0.056 0.004
32	56.8 2.3	3.4 0.2	7.0 0.5	8.9 0.9	10.9 1.2	0.073 0.013
38	37.7 1.1	3.2 0.2	7.1 0.4	9.3 0.7	14.2 2.5	0.078 0.009

Figure 4.24 Effect of temperature on mean particle size of emulsions formed by self-emulsification of 70%M812/30%T85 in distilled water.

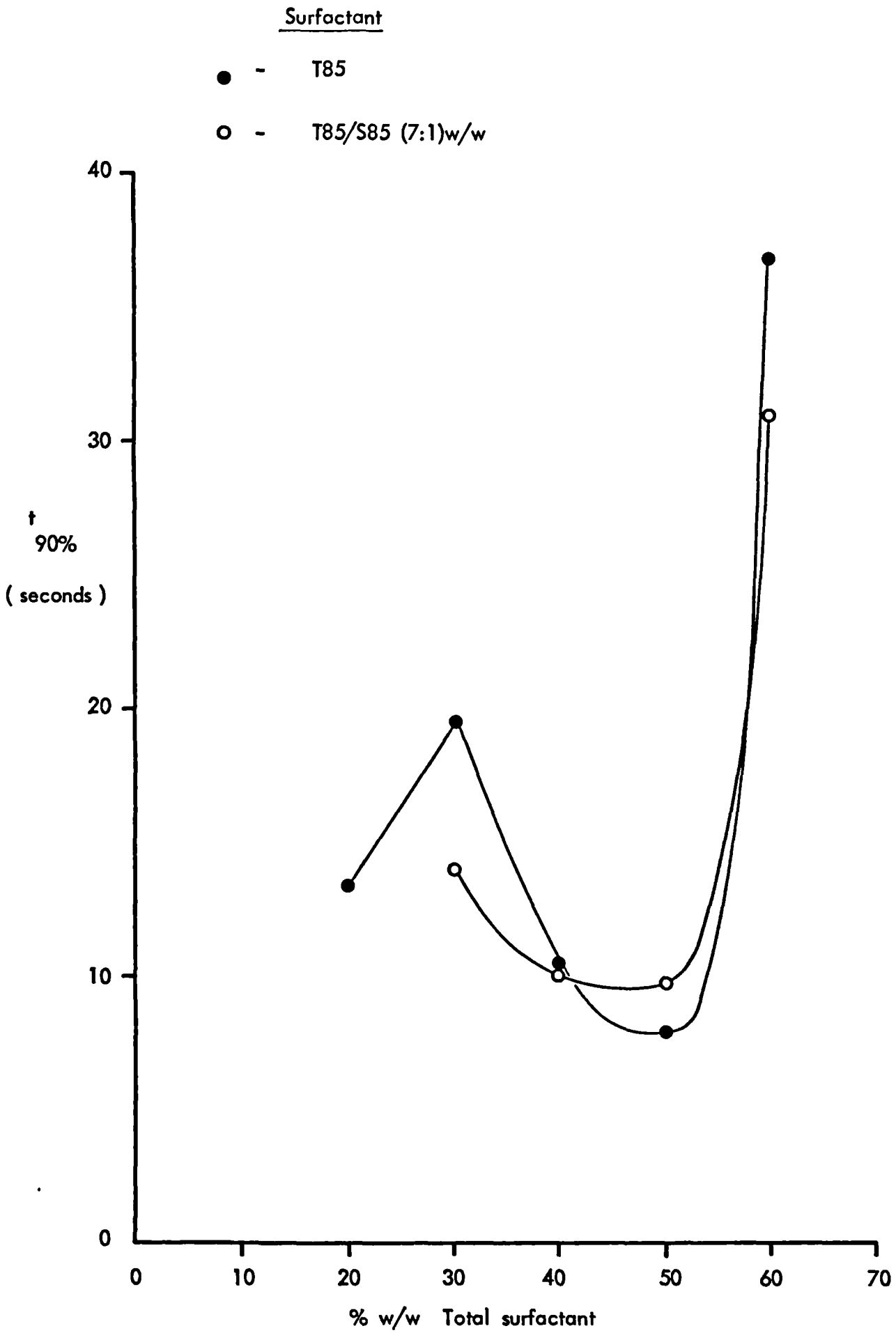


each addition. Below 15°C, after the I_o phase had become saturated with water, lamellar liquid crystal began to separate out forming the biphasic mixture II_{o+LC}. This was analogous to the behaviour of mixtures containing >50%T85 at 25°C. The result in each case was Type C emulsification behaviour. Type B beahviour was observed when the temperature of the 70%M812/30%T85 mixture was between 25° and 40°C, close to the CST and when the I_{LC3} phase could form. The quality of the emulsions improved as the temperature approached 40°C corresponding to increased prominance of the I_{LC3} phase and the low interfacial tensions close to the CST (the phase inversion region). The minimum particle size occured just below the CST and seemed to correspond with optimum conditions for liquid crystal formation. Certainly at temperatures above the CST emulsion quality began to deteriorate. By reference to Figure 4.14 it can be seen that above 60°C the phase behaviour of the 70%M812/30%T85 mixture is comparable to that of mixtures containing less than 15%T85 at 25°C (Fig. 4.16), which also exhibited Type A behaviour. Indeed it would seem that the efficiency of emulsification of M812/T85 mixtures can be predicted by reference to the appropriate phase diagram. For example one might predict that emulsification of the 50%M812/50%T85 would improve as temperature increased towards 60°C (towards the CST; when the mixture would have Type B character), but that quality would deteriorate above 80°C (above the CST; when the mixture would have Type A character). The above suggestions were confirmed

experimentally. Alternatively by referring to Figure 4.16 one might predict that no improvement in self-emulsification of the 85%M812/15%T85 mixture could be gained by raising the temperature above 25°C (because its phase behaviour at equilibrium corresponded to a Type A mixture over the range 25-80°C). The latter prediction was also confirmed experimentally.

Figure 4.25 shows $t_{90\%}$ values for M812/T85/S85 mixtures at 37°C. This temperature is of particular interest to the current study, being body temperature. Figure 4.25 is interesting when compared to its relative at 25°C (Figure 4.17). The two curves of M812/T85 mixtures are of similar shape but there was a shift in the minimum emulsification time at 37°C towards higher T85 concentrations. Furthermore, microscopical examination showed that the emulsion formed by the 20%T85 mixture at 37°C was poorer than that formed at 25°C. It appeared to have a lower $t_{90\%}$ but had a wide size distribution including some free oil. In other words at 37°C the 20%T85 mixture had Type A behaviour. Again, this change can be explained by reference to phase behaviour at equilibrium (Figure 4.15). At 37°C the 20%T85 mixture is above its CST and exhibits phase behaviour typical of a Type A mixture.By referring to the other phase diagrams (Figures 4.12 to 4.16) it can be anticipated that if the temperature at which emulsification is to take place is raised, then a higher proportion of T85 is required to produce a Type B mixture. (i.e. to allow emulsification

Figure 4.25 Self-emulsification times of M812/T85/S85 mixtures in distilled water at 37°C.



at or just below the region of phase inversion at the CST). Thus the minimum $t_{I\%}$ at 37°C occurs at higher T85 content (Fig. 4.25) than the minimum $t_{I\%}$ at 25°C (Fig. 4.17). Mixtures containing small amounts of S85 (using a T85/S85 7:1 mixture) were also examined at 37°C and plotted in Figure 4.25. Those mixtures featured in Figure 4.25 represent the mixtures which formed emulsions with Rel I_{100} values over 25. The behaviour of the mixtures containing S85 was similar to those using pure T85 as the surfactant but the absence of the 20% mixture (i.e. the Rel I_{100} value was less than 25) indicates that emulsion quality was poorer for the S85 mixtures (see section 4.1.3.2.). As discussed previously further increases in S85 content resulted in poorer quality emulsions and eventually Type A mixtures.

The changes in behaviour of non-ionic surfactant, oil and water systems as temperature increases are thought to be due to reduced hydration of the polyoxyethylene chains which are present as the hydrophilic moiety of the surfactant[116,118,123,137,159]. The cloud point of non-ionic surfactants in aqueous solution is the most obvious manifestation of this change in surfactant/water affinity. The theory has been extended to explain phase inversion phenomena and the regions of high oil and water solubilization near to the phase inversion temperature (PIT). The decrease in hydration of oxyethylene groups, as temperature is increased, appears to be gradual. Whilst the solubilization of water and oil for some surfactant

systems increases greatly close to the PIT, changes in emulsion properties may not be as dramatic, but may take place gradually as temperature is raised. The changes in efficiency of self-emulsification which have been observed during the current study did seem to occur gradually as the temperature was raised. The 70%M812/30% T85 mixture emulsified easily over the temperature range 25-40^{0}C. It was not necessary to emulsify at a precise temperature. For such systems it seems more appropriate to propose a range of temperature over which phase inversion occurs. Similar observations have been reported by Sherman and co-workers[133] as a result of their studies on PIT using differential thermal analysis[132,182]. However it is possible that the gradual changes observed in the current study and in Sherman's studies (in which industrial Tweens and Spans were used) are a result of the diversity of surfactant molecules present in industrial materials. Gibbons[183] has used the term heterodisperse to describe such materials. Other authors have commented on the importance of molecular weight distribution of non-ionic surfactants[184]. General behaviour can be predicted for industrial materials but phase changes may be less precise. For instance, for materials comprising a wide variety of molecules the cloud point is usually lowered but the solubilization region in the non-aqueous phase raised[103] (when compared with a more pure material of similar general structure). This may explain why the phase inversion region is less distinct for emulsions stabilized with 'Tweens' and 'Spans'.

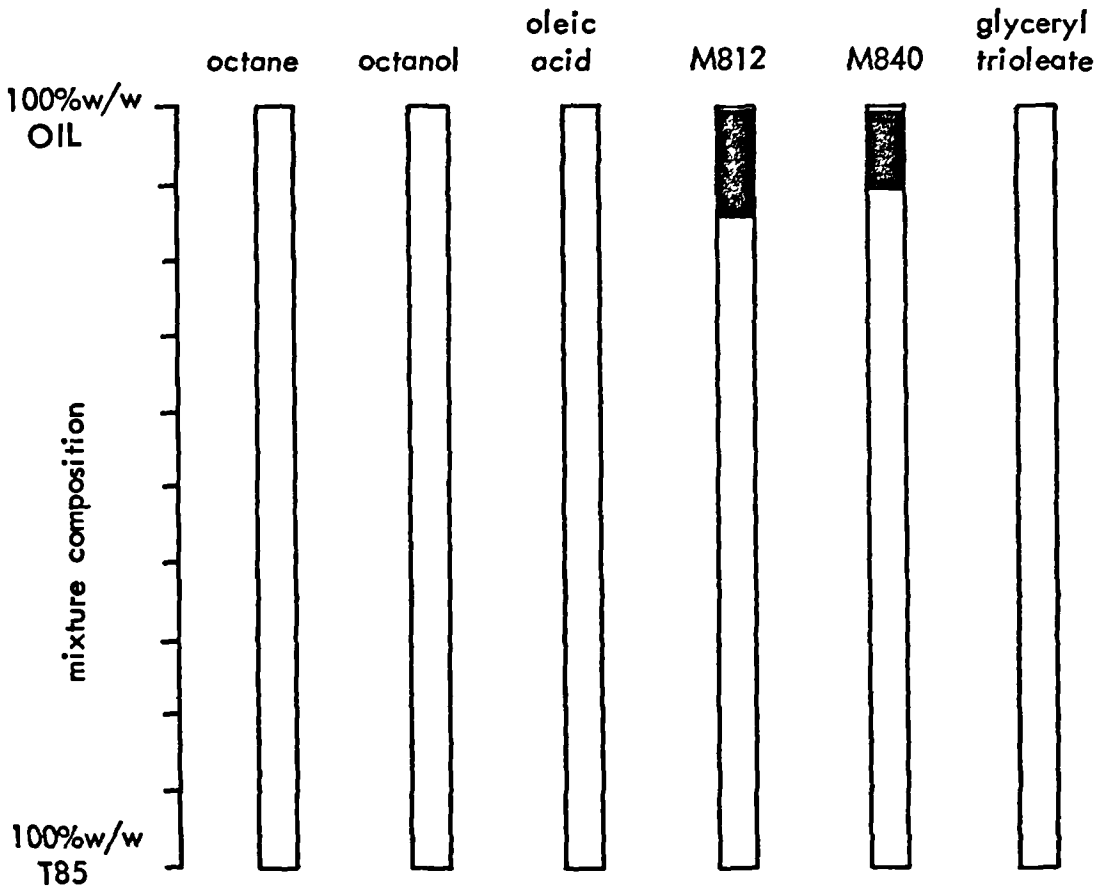
4.2. THE EFFECT OF OIL IDENTITY ON EMULSIFICATION OF OIL/T85 MIXTURES IN DISTILLED WATER

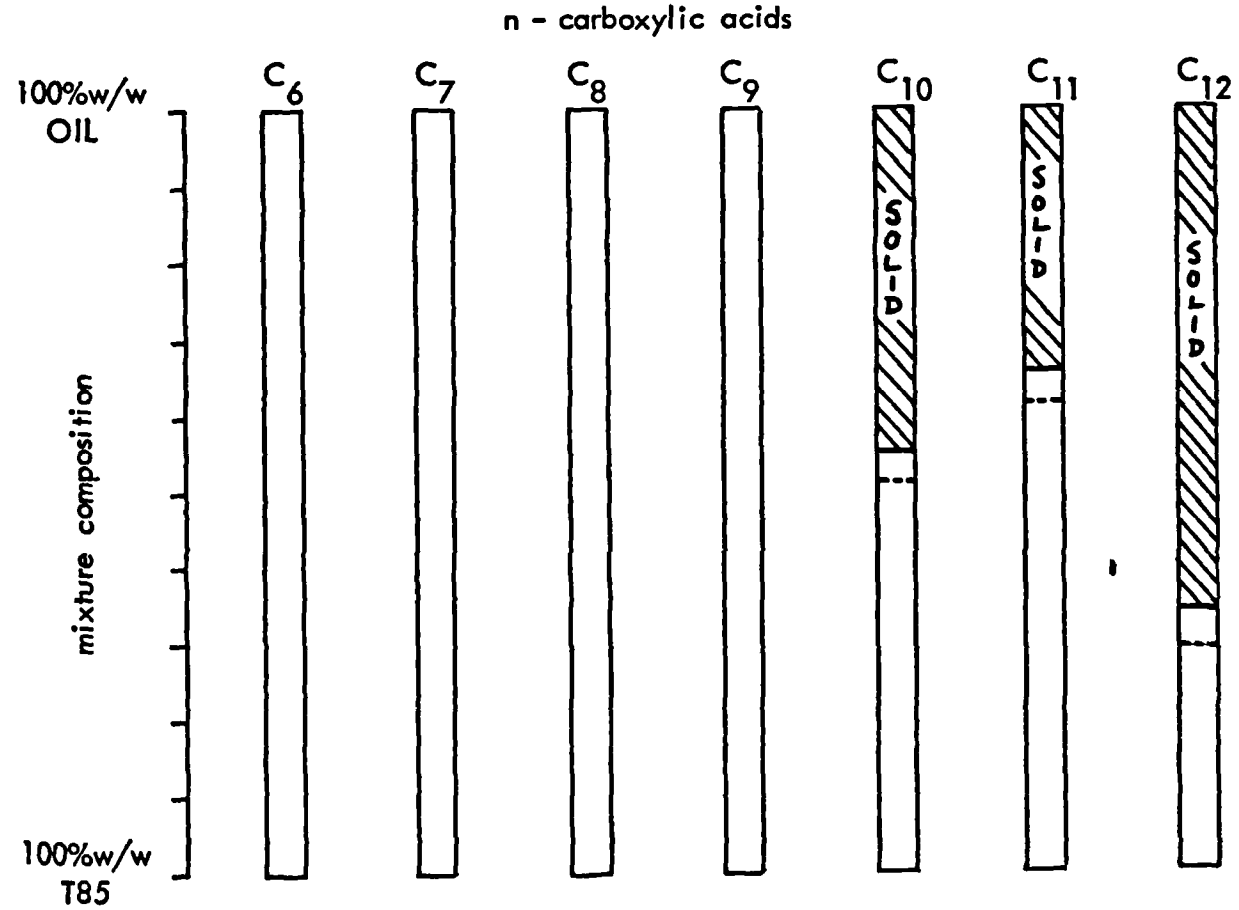
After Griffin's historic papers introducing the HLB concept [99-100] other researchers began to notice that the identity of the oil phase was also important to the nature of oil/surfactant/water mixtures. Ohba measured "required HLB" values for various oils[185] and Shinoda reported differences in the solubility of non-ionic surfactants in hydrocarbons[186]. Since then many authors have described differences in emulsion properties or phase behaviour as a result of variation in oil identity[120-122,164,187,188]. The importance of the oil type is not surprising; this general term covers a wide variety of materials.

An investigation of a series of oils was of interest to the current study for two main reasons. Firstly by examining a series of related materials of different density and viscosity an indication of the importance of these properties to emulsification might be observed. Secondly by relating the properties of other oils to M812 one might be able to conclude which properties particular to M812 aid self-emulsification.

The oils examined and their phase behaviour when mixed with T85 are shown in Figure 4.26. Only the two Miglyols were partially miscible with T85. The three other C_8 oils of different polarities mixed in all proportions with the T85. The series of fatty acids varied extremely in viscosity. All were miscible with T85 but the C_{10}-C_{12}

Figure 4.26 Phase behaviour of oil/T85 mixtures at 25°C (shaded areas represent diphasic mixtures)





acids, which were solid at 25°C, formed crystalline mixes at high fatty acid concentrations. Glyceryl trioleate was also miscible with T85 despite the likelihood that it was less polar than the Miglyols. The miscibility of glyceryl trioleate is probably explained by its low moisture content.

The viscosities of M840/T85 mixtures were lower than those of M812/T85 but deviated from ideal behaviour in a similar fashion (Figure 4.27) suggesting that T85 formed similar micelles in both materials. Figure 4.28 shows the viscosities of the other oil/surfactant mixtures emphasising the large differences at low T85 concentrations. All the oil/surfactant mixtures showed deviation from ideal behaviour but the shapes of the curves are different suggesting that the nature of 'micellization' probably depended on the chemical nature of the oil. Octane mixtures had very low viscosities and those of glyceryl trioleate were particularly high. Densities of the same mixtures are plotted on Figure 4.29. The two Miglyols were the most dense materials. The materials which were notable for low density were octanol and, more particularly, octane. The plots of density versus mixture composition were all non-linear but deviations from linearity were slight.

Figure 4.27 Viscosities of M812/T85 and M840/T85 mixtures at 25°C.

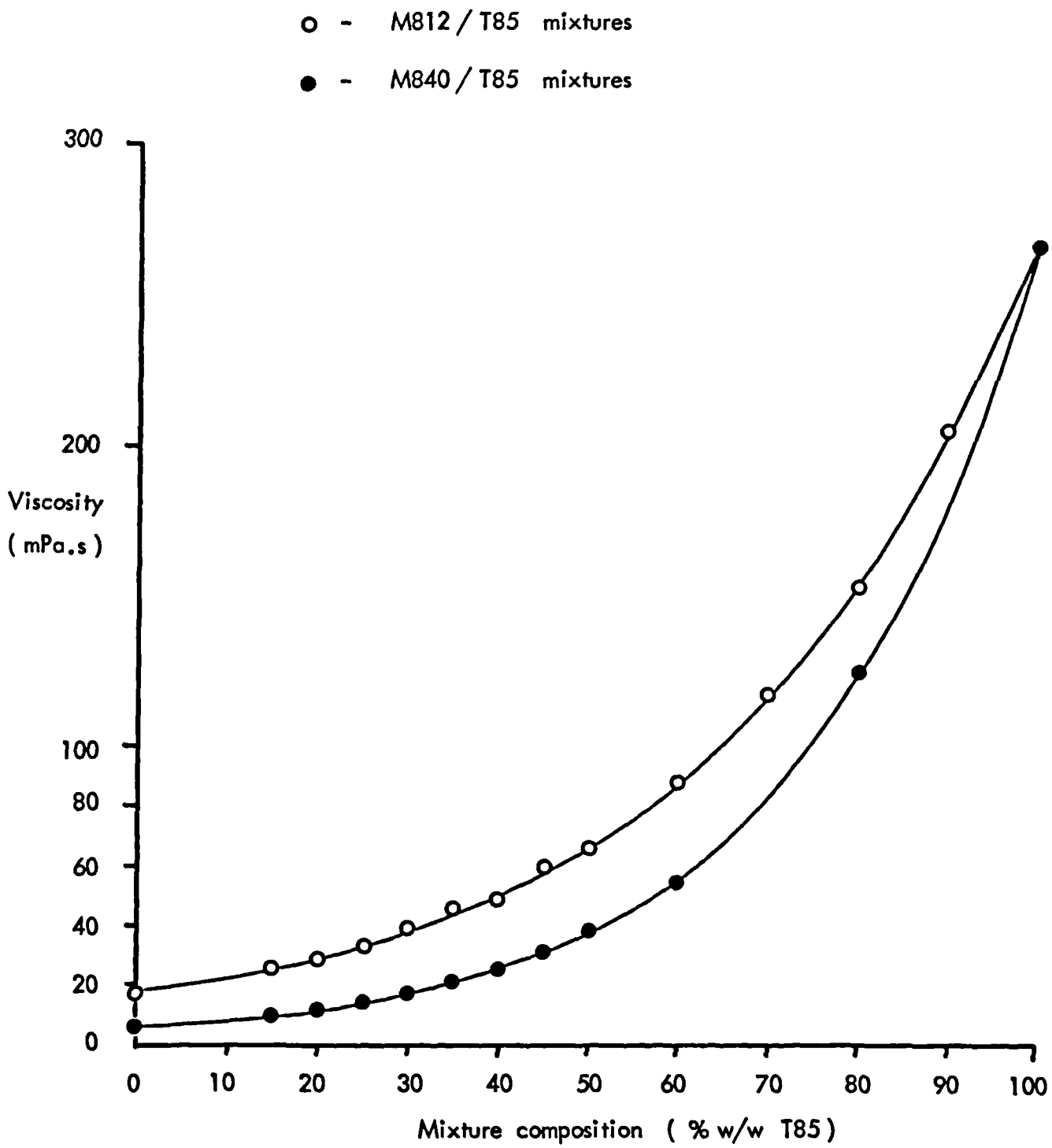


Figure 4.28 Viscosities of oil/T85 mixtures at 25°C.

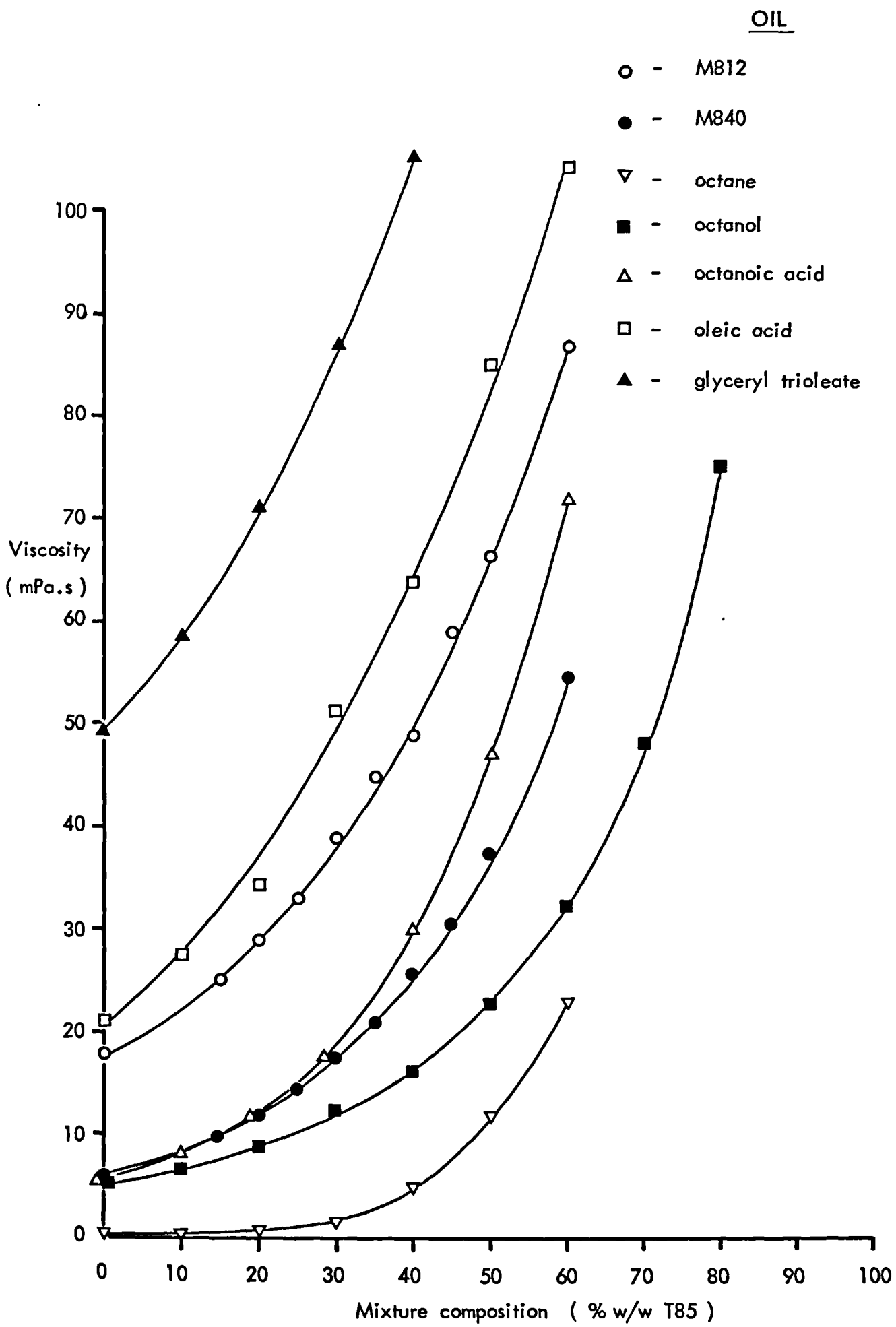
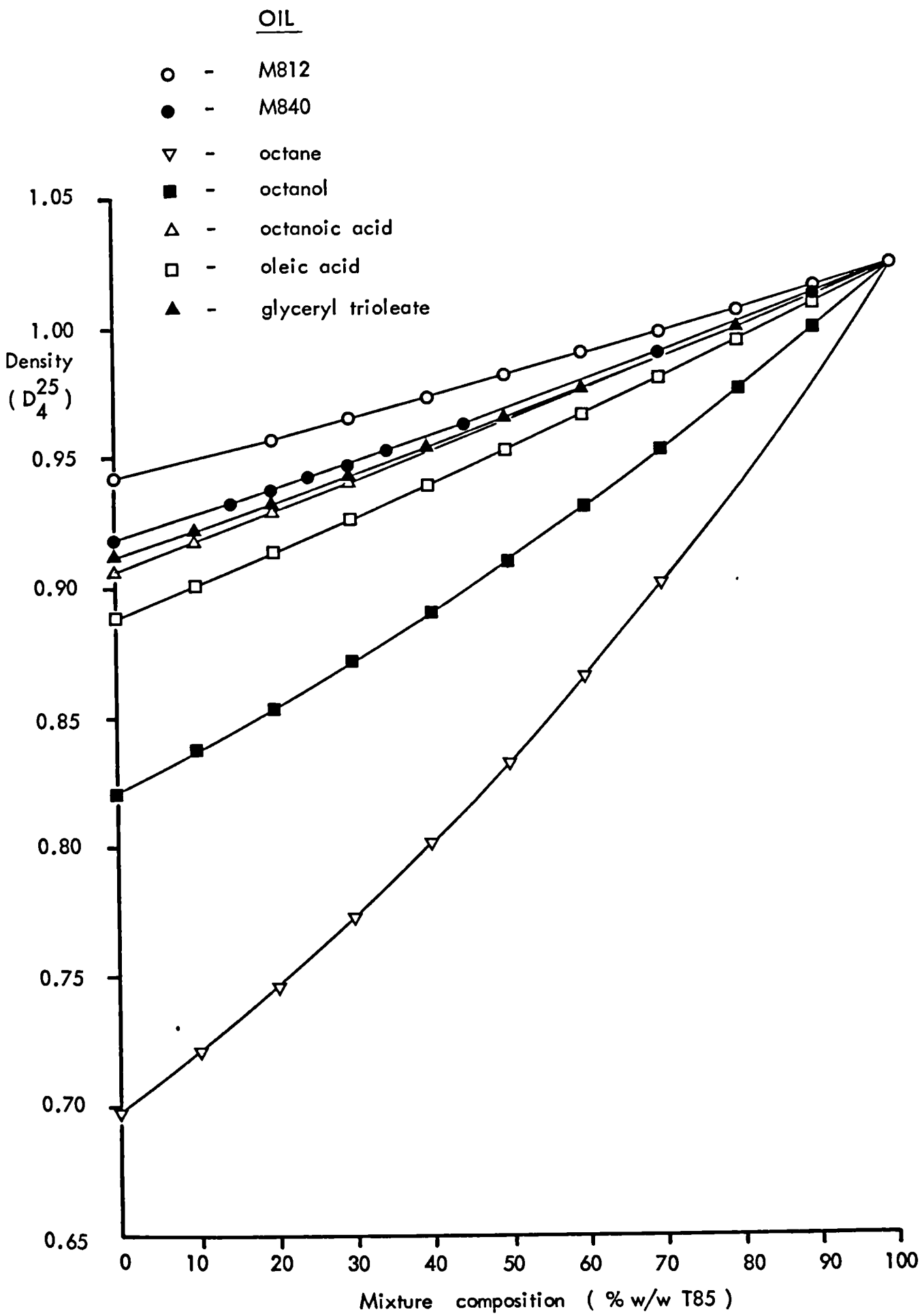


Figure 4.29 Densities of oil/T85 mixtures at 25°C.



Miglyol 840 had emulsification properties closely related to M812. The plots of $t_{I\%}$ versus surfactant concentration (Figure 4.30) and mean particle size of emulsions versus surfactant concentration (Figure 4.31) are analogous to Figures 4.17 and 4.18. Both plots have minimum values and, as in the case of M812 mixtures, the minimum particle size occured at a T85 concentration approximately 10%^{w}/w lower than the minimum $t_{I\%}$. The minimum values for $t_{I\%}$ were lower for the M840 mixtures than the M812 mixtures. Significantly the t_o values were lower, which may suggest that the differences were due to the lower density and viscosity of M840. This seems more likely in the light of the fact that the minimum values for particle sizes of emulsions were similar for both Miglyols. The changes in behaviour of M840/T85 mixtures as T85 concentration was increased again represented a transition between Type A to Type D mixtures. The difference between the two oils was that the transitions occured at different T85 concentrations. Infact the minima for $t_{I\%}$ and mean particle size for the M840/T85 mixtures both occurred at T85 concentrations 5%^{w}/w lower than corresponding minima for M812 mixtures. This slight shift of the curves towards lower T85 concentrations was general. M840/T85 mixtures containing less than 15%T85 were Type A (but the 15%T85 mixture resulted in a better quality emulsion than the corresponding 85%M812/15%T85 mixture). The Type B region for M840 mixtures extended from 20-35%T85 and Type C from 40-60% T85. Again, the shifts are approximately 5%^{w}/wT85. With

Figure 4.30 Self-emulsification times of M840/T85 mixtures in distilled water at 25°C.

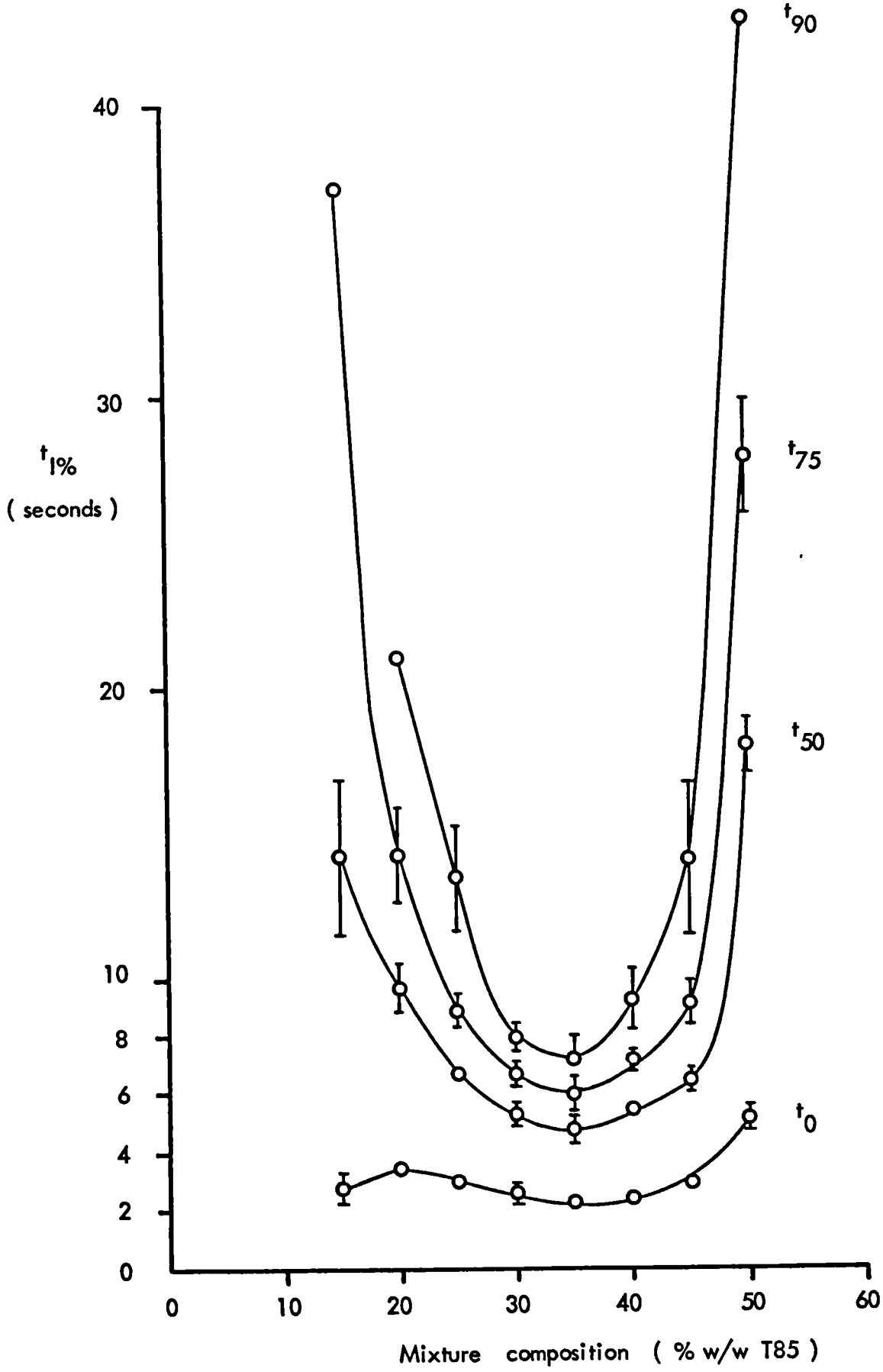
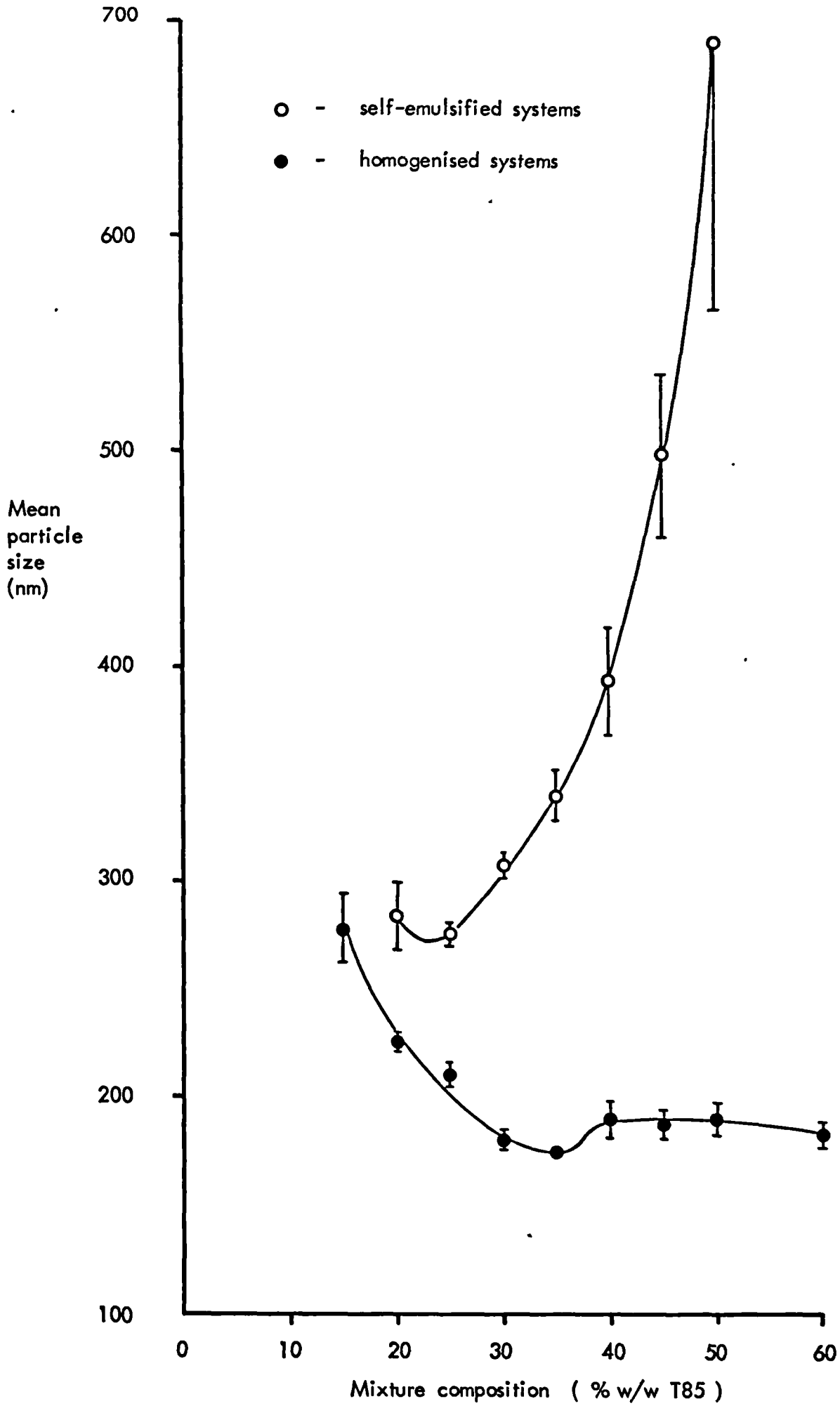


Figure 4.31 Mean particle sizes of self-emulsified and homogenised emulsions of M840/T85 mixtures in distilled water at 25°C.



respect to the equilibrium phase behaviour the difference between the two Miglyols was that the M812 caused a greater lowering of the CST at each particular concentration. Consequently the optimum conditions for emulsification occurred at a higher concentration of T85 for M812/T85 mixtures. This suggests that the M812 was a slightly more polar material than M840[117]. The homogenised emulsions of M840/T85 mixtures (Figure 4.31) had very similar particle sizes to emulsions formed by homogenisation of M812/T85 mixtures reaching a low plateau of about 180nm above 30%T85.

Self-emulsification of other oil/T85 mixtures was examined at 25°C selecting the following T85 concentrations; 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 80%^{w}/w. Emulsification was very poor in every case examined. The value of Rel I_{100} was less than 10 for each mixture examined and for most was well below 5. The differences between various oils will be discussed below but it is important to stress that only Miglyol/T85 mixtures produced fine emulsions by self-emulsification at 25°C.

The effects of viscosity and density on emulsification of oil/T85 mixtures are only likely to be significant at low surfactant concentrations when considerable energy is required to disperse the oil/T85 mixtures. Self-emulsification of octane/T85 mixtures was very poor despite their low viscosities and densities. It is

reasonable to suggest that the comparative efficiency of Miglyol/T85 mixtures was due to particular effects of molecular interaction as exemplified by phase studies and that when such conditions were met the effects of viscosity and density of the mixtures were negligable.

The interesting question was whether or not the poor self-emulsification of the alternative oil/surfactant mixtures could be explained by phase behaviour. With this in mind a selection of phase studies was undertaken.

Figures 4.32 to 4.35 demonstrate the effect of four oils on phase behaviour. 50%T85 in oil mixtures were compared with the equivalent M812 mixture (Figure 4.12). Octanoic acid (Fig. 4.33) and Octanol (Fig. 4.34) phase diagrams showed little change with temperature. In both cases the extent of water solubilization in the I_o phase was insensitive to temperature change in contrast to the phase diagram of the M812 mixture. As water was added to the saturated I_o phase of octanoic acid or octanol mixtures phase separation occurred resulting in a water-in-oil emulsion. This phase behaviour might be explained by the more polar character of octanoic acid and octanol due to their aliphatic hydroxyl groups. T85 was effectively a water-in-oil emulsifier for octanol/water or octanoic acid/water mixtures. Or, in other words, the CST was lowered to the extent that it did not appear on the phase diagram. Lower T85 concentrations in

Figure 4.32 Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% Octane.

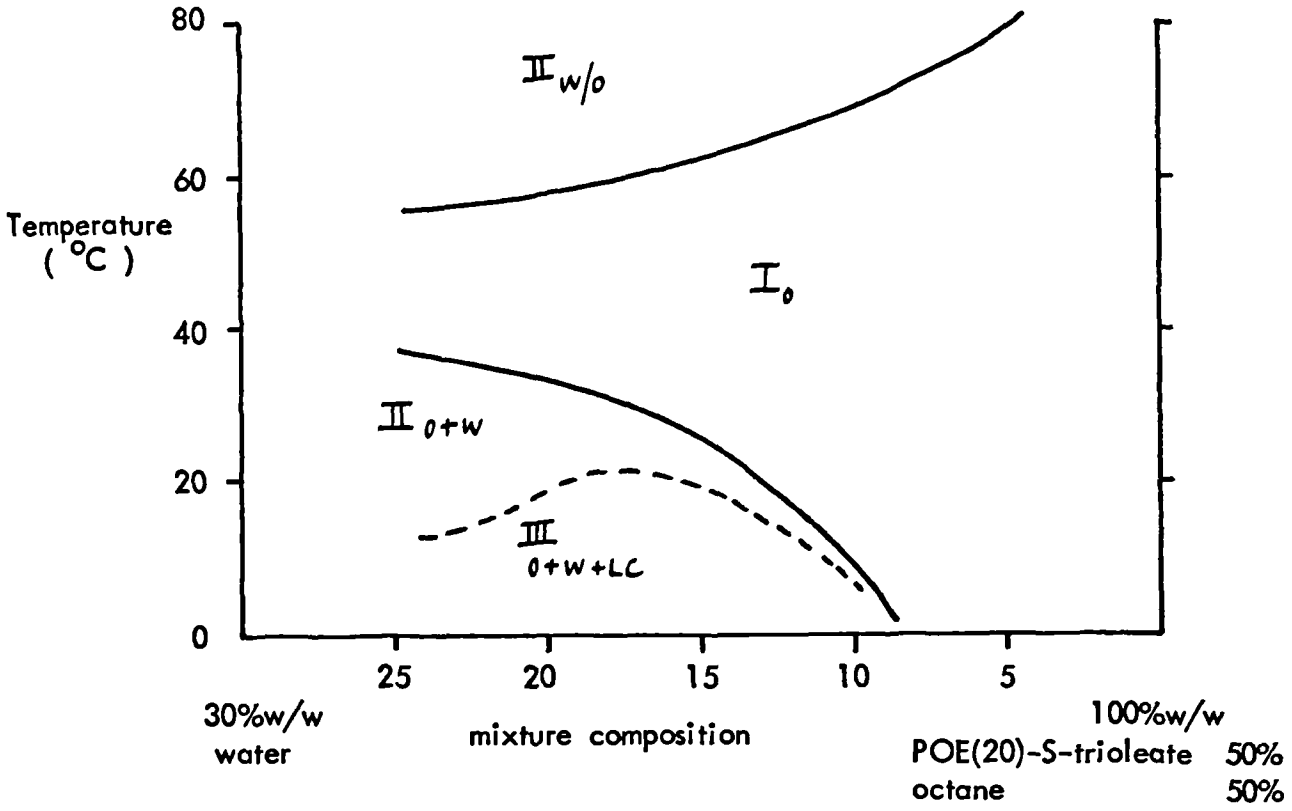


Figure 4.33 Phase diagram of water and the mixture 50% POE-(20)-S-trioleate and 50% Octanoic acid.

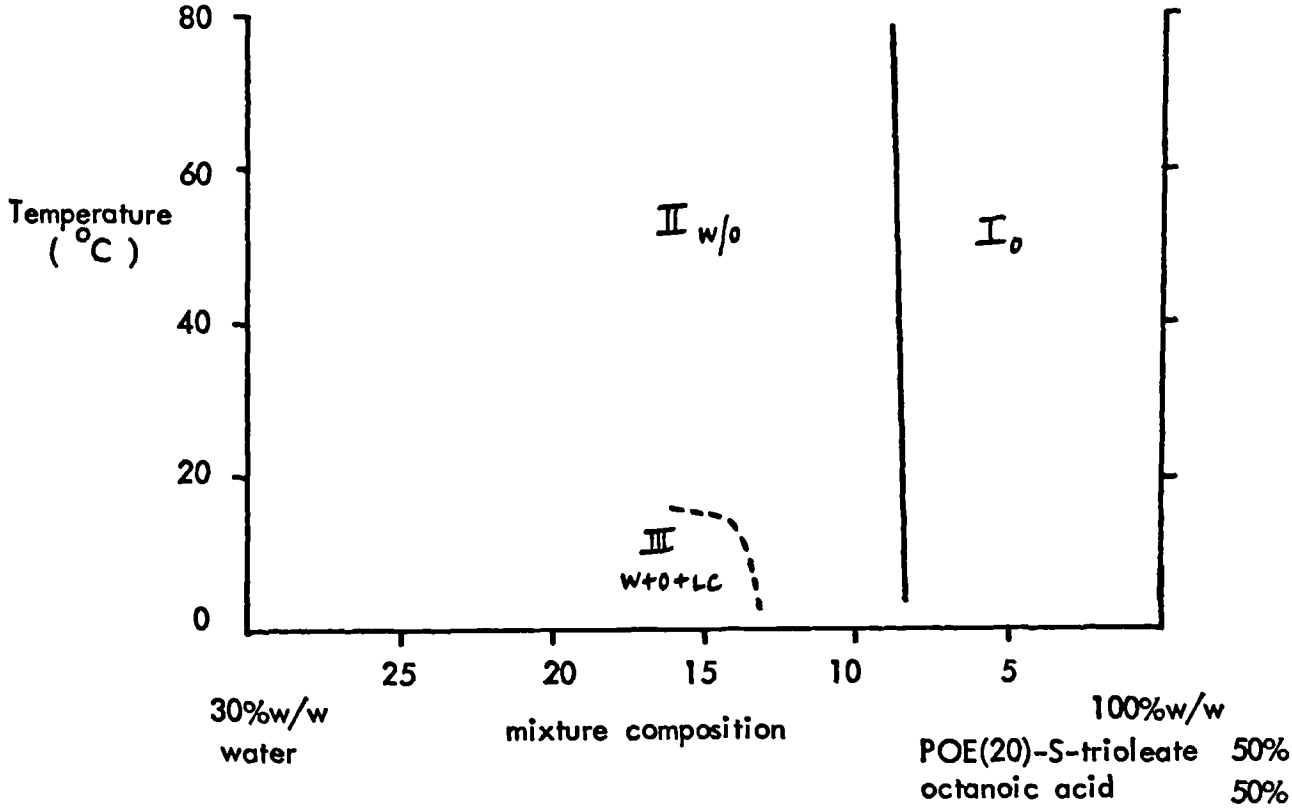


Figure 4.34 Phase diagram of water and the mixture 50% POE(20)-S-trioleate and 50% Octanol

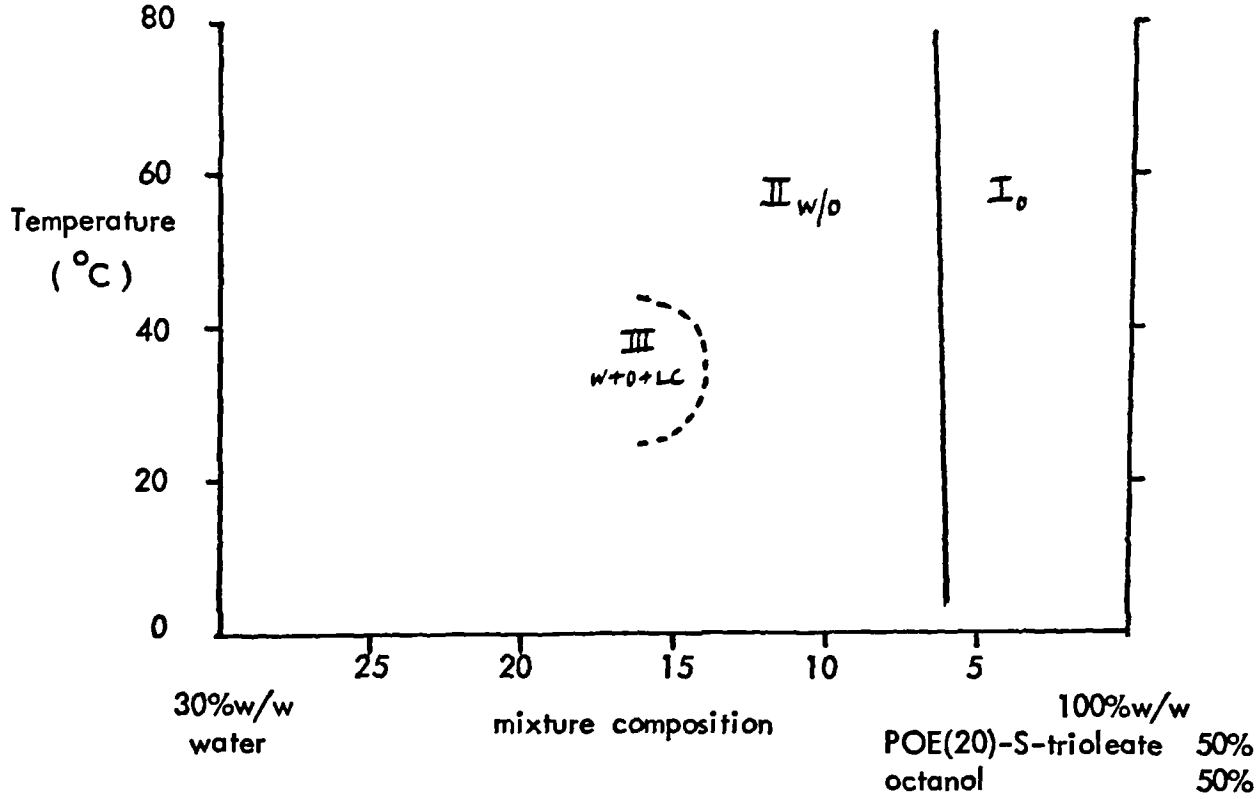
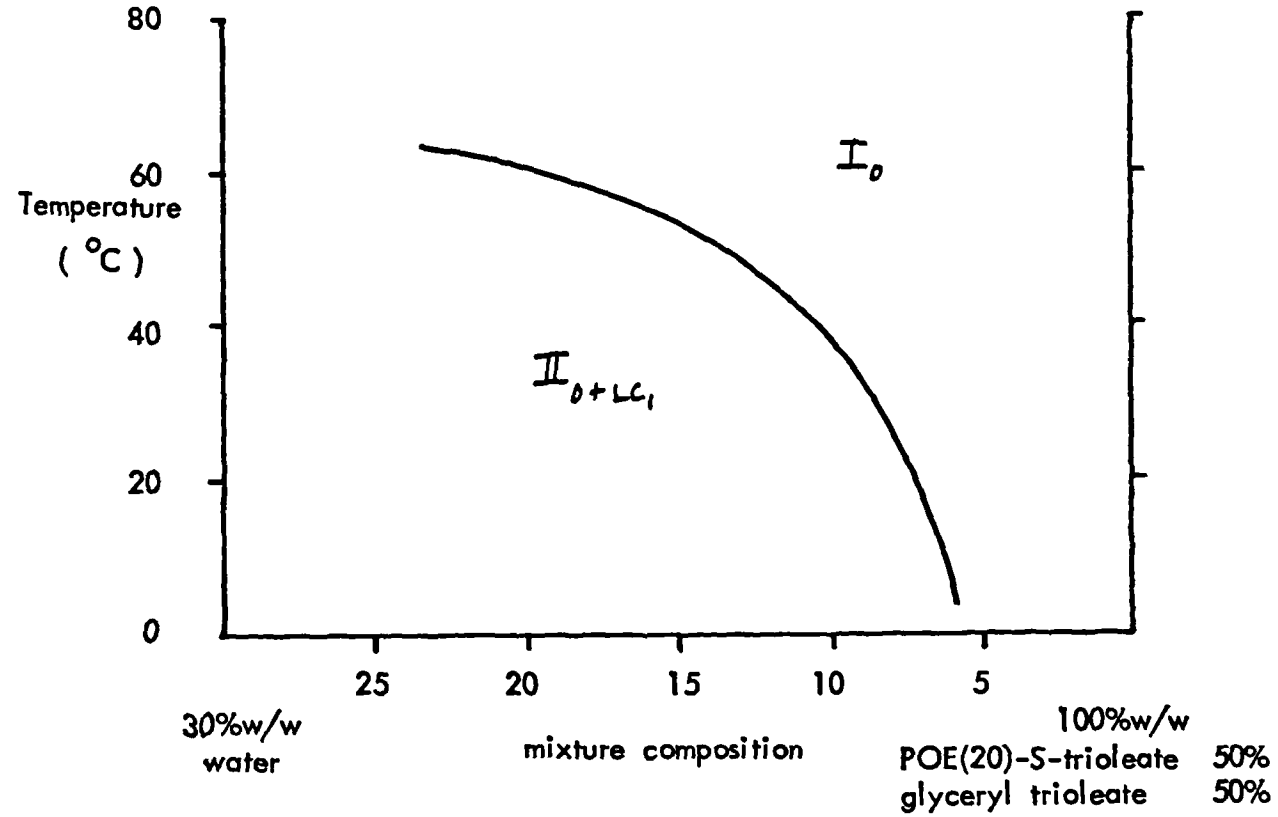


Figure 4.35 Phase diagram of water and the mixture 50% POE(20)-S-trioleate and 50% glyceryl trioleate.



octanoic acid or octanol produced similar phase diagrams with water, as was expected. Therefore poor emulsification resulted for these two oils and also for all the other fatty acids (C_6 - C_{12} and oleic acids) due to their comparative polar nature.

The two oils, octane and glyceryl trioleate, had more interesting properties. The glyceryl trioleate mixture (Figure 4.35) had a large region of water solubilization (I_o) which appeared to approach a critical temperature higher than that of the M812 mixture. This, again, is in agreement with Shinoda's work being indicative of the more non-polar nature of the trioleate (due to its longer hydrocarbon chains). When the I_o phase of the glyceryl trioleate mixture was saturated with water a liquid crystalline phase began to form if more water was added. This behaviour was similar to that of M812 mixtures containing high concentrations of T85 when the latter mixtures were diluted with water. For M812 mixtures the above phase behaviour was typical of Type C mixtures. Indeed similar emulsions resulted from the glyceryl trioleate mixtures. Infact the glyceryl trioleate mixtures produced better quality emulsions than any oils other than the Miglyols. One might have expected that by decreasing the T85 concentration one could produce conditions for efficient self-emulsification, i.e. produce Type B mixtures. However, this did not occur with glyceryl trioleate as shown by the particle

size data (Table 4.5). Emulsions were polydisperse with mean particle sizes between 1 and 2μm. Mixtures containing less than 10%T85 produced coarse emulsions with some free oil.

The 50%T85 in octane mixture also showed an I_o region which was sensitive to temperature and the critical temperature was below that of the 50%M812/50%T85 mixture (Figure 4.32). Again, this oil seemed to have some of the properties of phase behaviour characteristic of the M812 mixtures. However despite the fact that the CST was similar for octane/T85 mixtures and Type B M812/T85 mixtures the emulsification of 50%T85 in octane was very poor. The notable difference between the octane and M812 phase diagrams is the absence of liquid crystal on the octane phase diagram. Traces of liquid crystal were observed for octane mixtures at lower temperatures and there was a corresponding increase in emulsion quality (although the quality was still poor) at temperatures under 10^{o}C. This observation suggests that formation of liquid crystal was important for self-emulsification of octane and Miglyol mixtures.

It would be interesting to investigate the effect of carbon chain length for a series of triglycerides on phase behaviour and self-emulsification. Unfortunately the series would be difficult to prepare in sufficient quantities. Such a study may isolate the crucial

Table 4.5. Quality of emulsions formed by self-emulsification of glyceryl trioleate/T85 mixtures in distilled water at 25°C.

MIXTURE COMPOSITION %w/w		PARTICLE SIZE (nm)		P.I.
T85	Glyceryl trioleate	Mean	S.D.	
10	90	> 3000	-	-
20	80	2863	57	9
30	70	1369	273	9
40	60	1905	531	9
50	50	1539	532	9
60	40	1367	48	9

properties of self-emulsifying mixtures.

If sufficient energy was available for emulsification it was possible to form fine emulsions with all the oils discussed above as shown by the particle sizes of homogenised emulsions of fatty acid/T85 mixtures (Table 4.6). Although examination of Table 4.6 indicates that larger particle sizes resulted as the polarity of the oil increased i.e. as T85 became less suitable for o/w emulsification.

Table 4.6. Quality of homogenised emulsions of carboxylic acid/T85 mixtures prepared in distilled water at 25°C.
(determined 24 hours after preparation)

Carbon chain length	MIXTURE COMPOSITION %w/w carboxylic acid	T85	Mean particle size (nm)	P.I.
C_8	90	10	177	4
	80	20	138	4
	70	30	132	3
	60	40	136	4
	50	50	145	2
	40	60	147	2
	20	80	138	5
C_6	70	30	185	7
	50	50	161	7
C_7	70	30	149	4
	50	50	191	7
C_9	70	30	187	2
	50	50	147	4
C_{10}	50	50	137	2
C_{11}	50	50	141	2

4.3. A COMPARISON OF PHYSICAL PROPERTIES OF ALTERNATIVE TWEEN SURFACTANTS

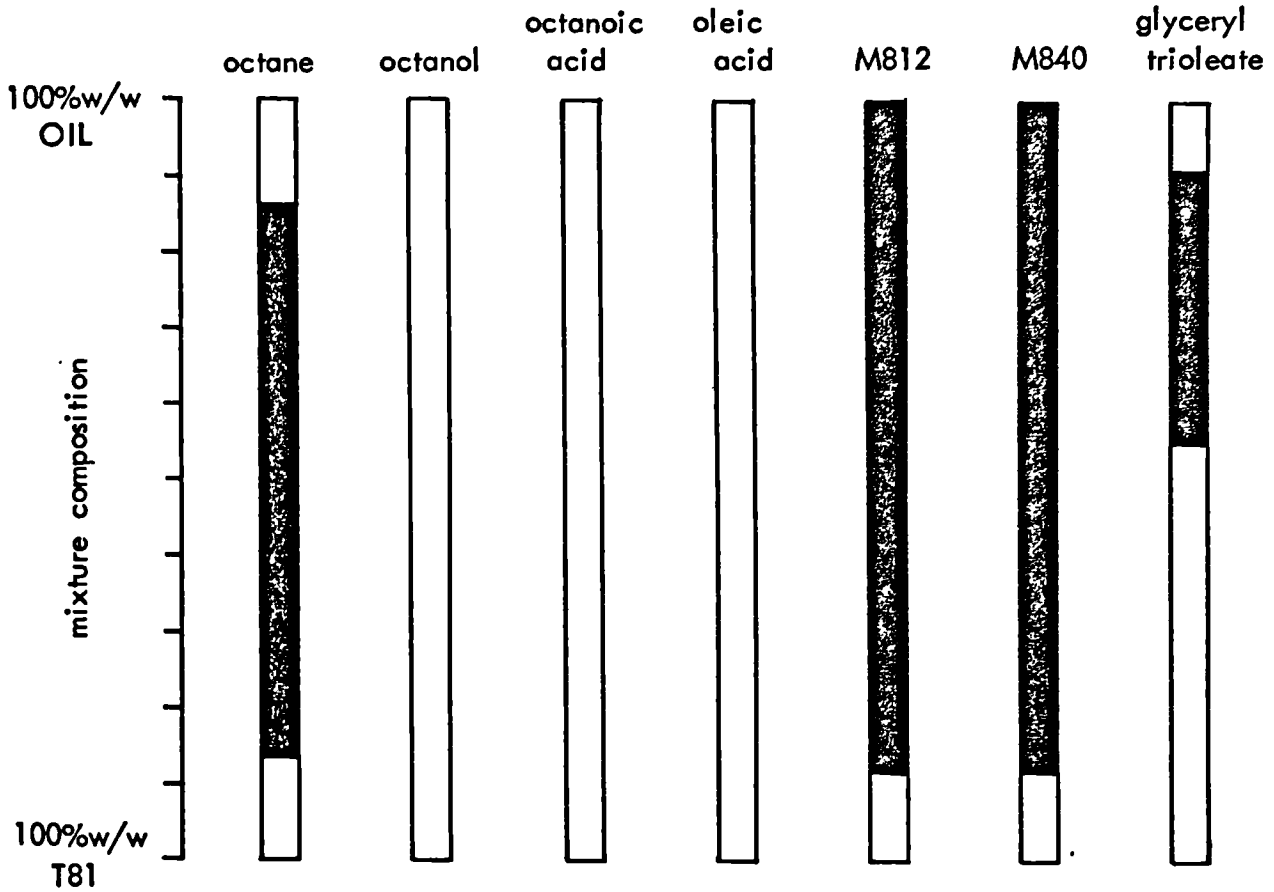
Two other Tween products derived from oleate esters of sorbitan were examined in order to compare their properties with T85.

Figure 4.36 represents phase behaviour of the industrial materials with a series of oils. The more non-polar materials, octane, glyceryl trioleate and the Miglyols showed diphasic regions with both T81 and T80. The oil/T81 mixtures were not capable of solubilizing as much water as corresponding oil/T85 mixtures. This may have been due to differences in the abilities of the surfactants to form micelles. As expected the emulsification of the polar oils by T81 was very poor and therefore T81 was of no further interest for the particular oils being used.

The diphasic mixtures formed by T80/oil mixtures were not surprising because T80 is considerably more hydrophilic than T85 (HLB-15.0, c.f. T85 HLB - 11.0). The extent of water solubilization tends to be lower for lipid solutions of more hydrophilic surfactants and, the PITs of oil/water/hydrophilic surfactant systems are higher[120,124].

At ambient temperatures, when non-aqueous micelles of T80 in M812 were saturated with water, M812/T80/water mixtures separated into a phase rich in water and surfactant and a second phase composed substantially of oil.

Figure 4.36 Phase behaviour of oil/T81 and oil/T80 mixtures at 25°C.
(shaded areas represent diphasic mixtures)



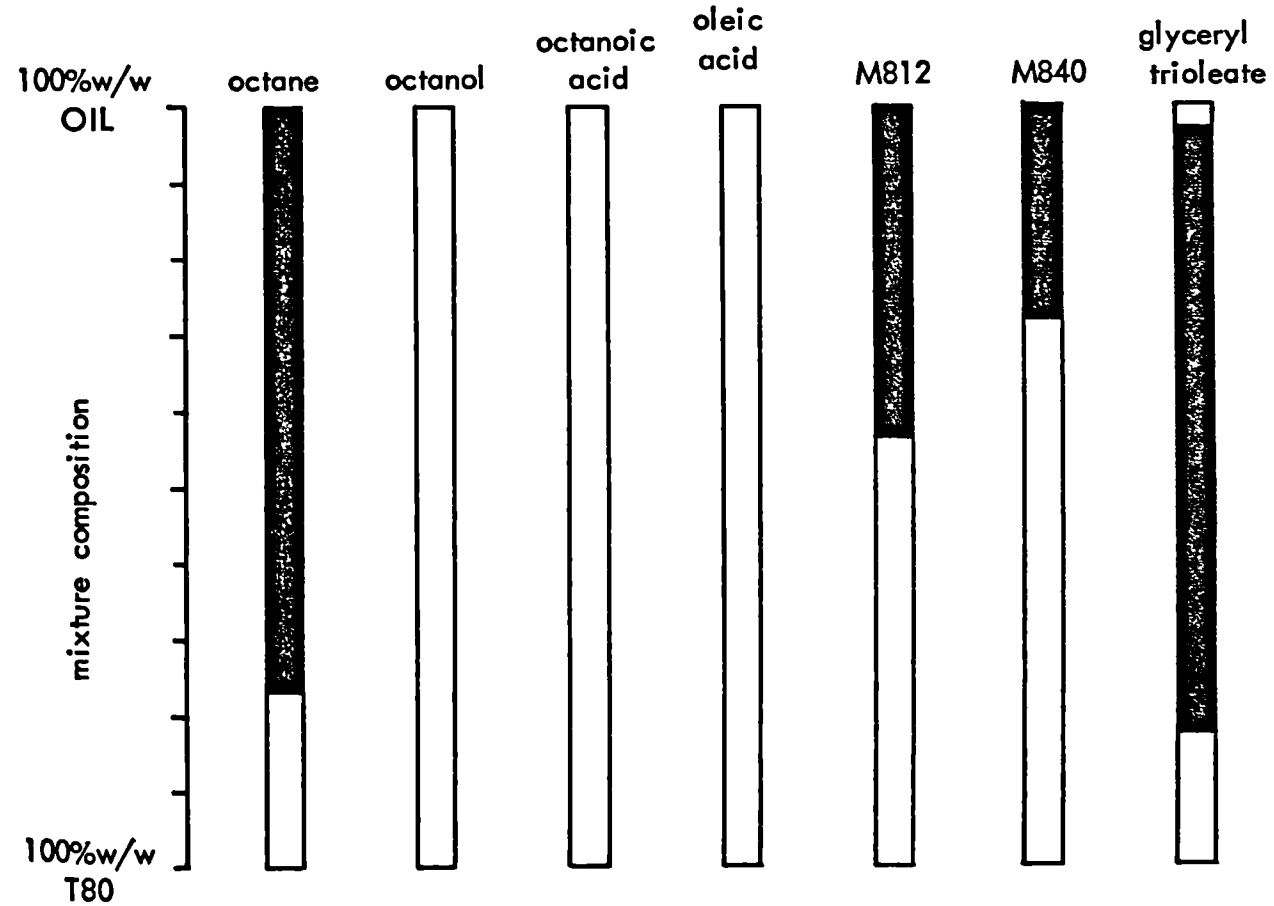


Figure 4.37 shows the regions of phase separation (II) and cosolvency (I) for Miglyol/T80/S85 mixtures at 25°C. The self-emulsification of mixtures within regions of cosolvency was investigated.

The phase diagram of water and POE-(20)-S-mono-oleate (the surfactant present in T80) was typical of a non-ionic surfactant with an HLB greater than 13[112,189] (Fig. 4.38). There was a large single phase region; between 30° and 80°C the surfactant and water were miscible in all proportions forming clear isotropic mixtures. Above 80°C the surfactant separated from solution depending on the mixture composition. This was the cloud point of the surfactant (when micelle formation, and hence solubility, is no longer thermodynamically favourable due to decreased hydration of the oxyethylene moiety of the surfactant). There was a small region of semi-solid liquid crystalline mixtures at temperatures lower than 20°C and a transitionary II_{w+LC} region between the liquid crystal and micellar regions. By comparing Figure 4.38 with the equivalent phase diagram for T85 (Figure 4.8) it can be seen that the additional oleate chains present in T85 make the materials very different in character. A phase diagram (0-80°C) of water versus 50%M812/50%T80 was completely diphasic but for a very narrow band of cosolvency (I_o) close to the oil/surfactant axis. The PITs of emulsions formed with T80 were much higher than 80°C.

Figure 4.37 Phase behaviour of Miglyol/T80/S85 mixtures at 25°C

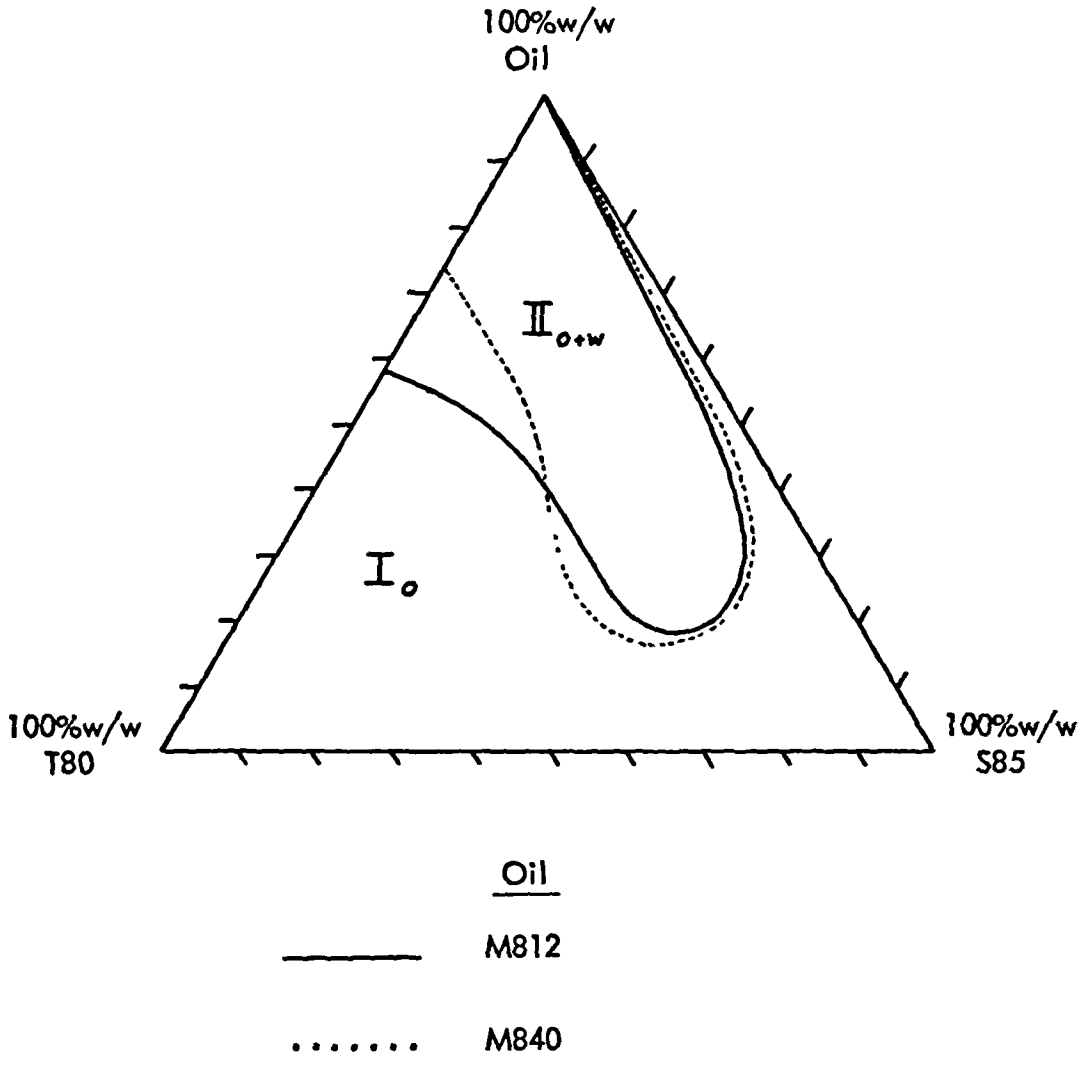
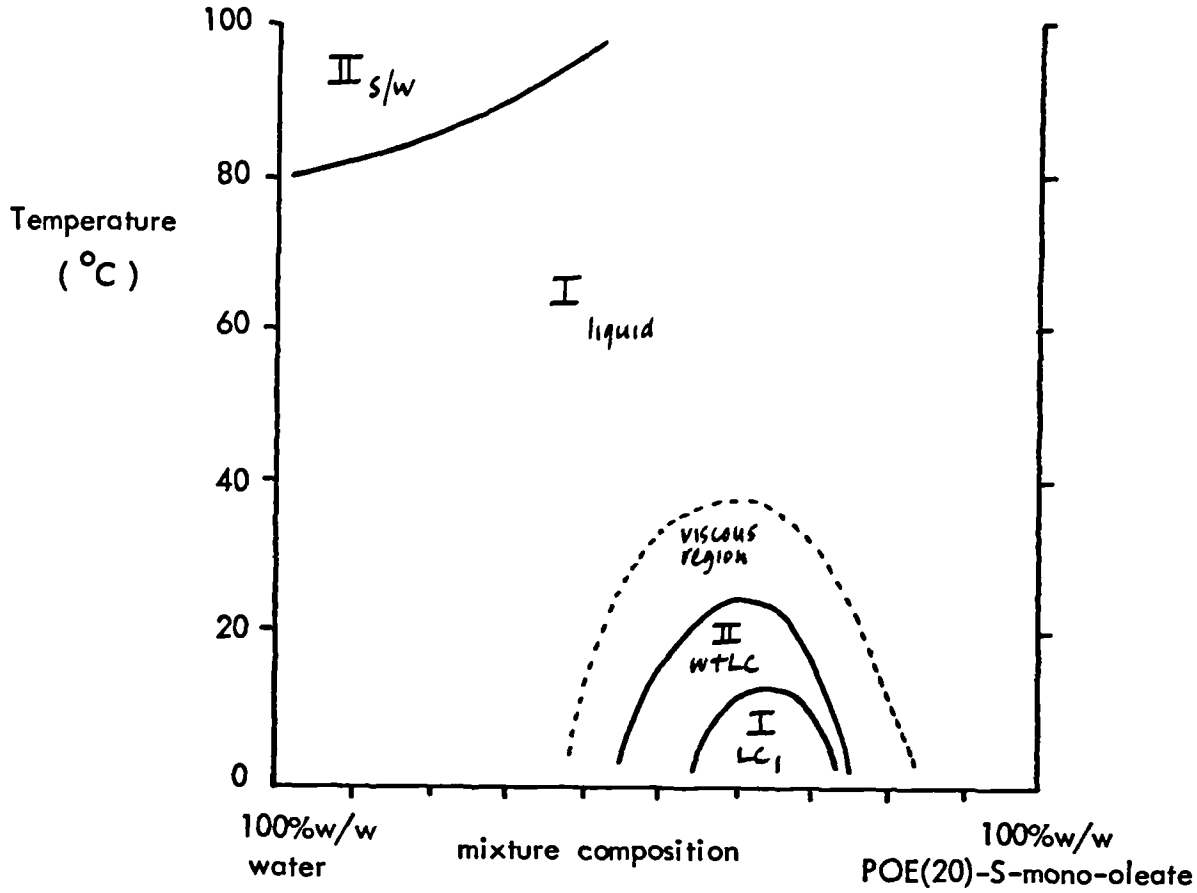


Figure 4.38 Phase diagram of water and POE(20)-S-monooleate.



Despite the large differences in phase behaviour between T80/Miglyol mixtures and corresponding T85 mixtures, some self-emulsification of T80/Miglyol mixtures was observed. Figure 4.39 represents the self-emulsification of M840/T80 mixtures. Close to the phase boundary emulsification did not occur efficiently but as the T80 concentration increased the mean particle size of resultant emulsions decreased steadily. At the same time the emulsification times, $t_{I\%}$, lengthened. The values of $t_{I\%}$ were far longer than for corresponding T85 mixtures and continued to rise as surfactant concentration was increased above 50% although the standard deviations were large. Self-emulsification of M812/T80 mixtures was directly analogous (Figure 4.40) to that of M840 mixtures.

The influence of S85 on M840/T80 mixtures was also examined. Figure 4.41 shows that there was little difference in emulsification rate but that as the S85 concentration increased there was a rise in mean particle size. S85 did not aid dispersion.

It is likely that the self-emulsification of T80 mixtures was facilitated by diffusion of the T80 molecules across the oil/water interface due to its high affinity for the excess aqueous phase. Emulsification of the oil would then have taken place either by way of mechanical disruption of the interface caused by strong diffusion of the T80 or by a "diffusion and stranding" process

Figure 4.39 Self-emulsification of M840/T80 mixtures in distilled water at 25°C.

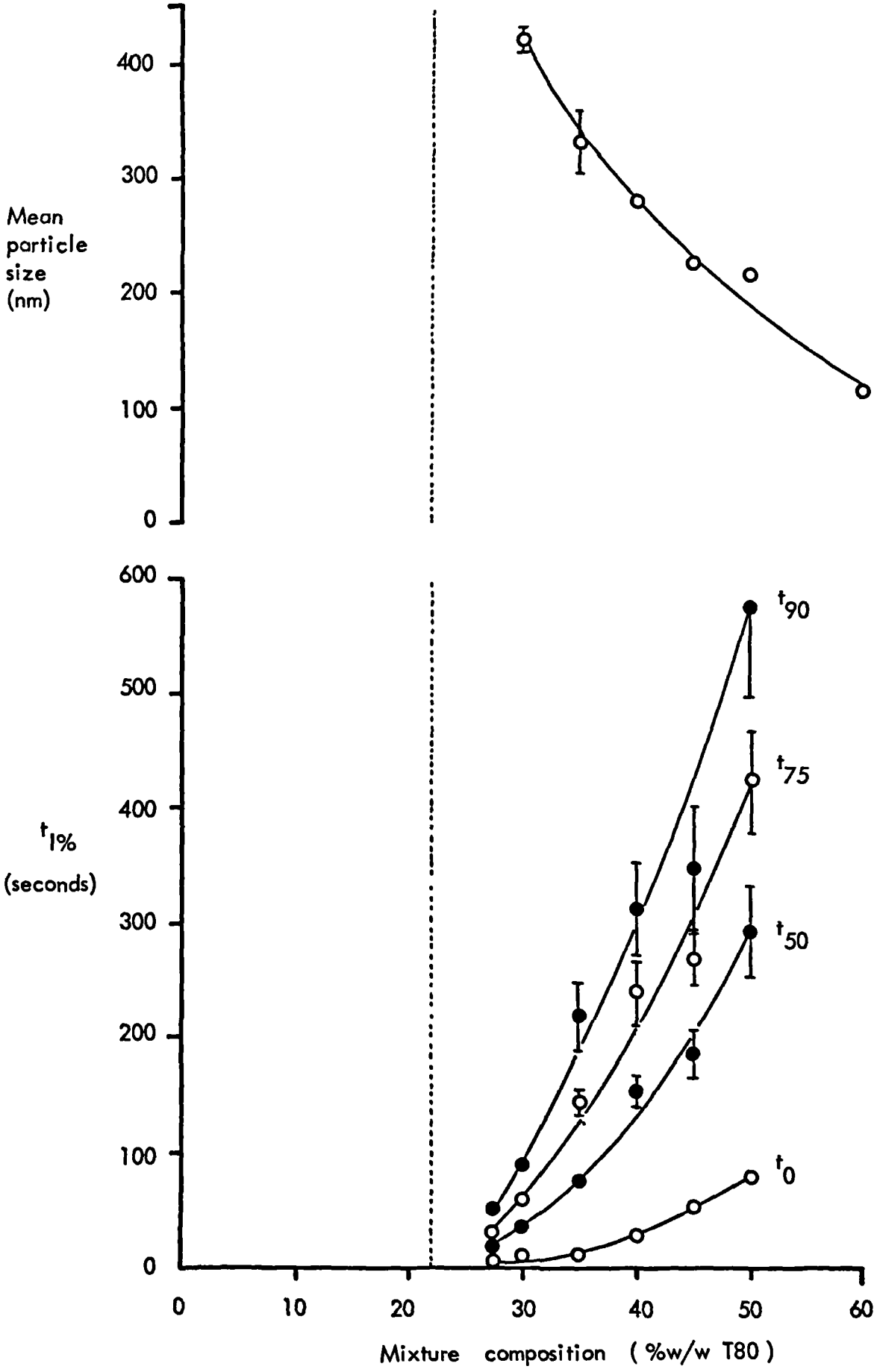


Figure 4.40 Self-emulsification of M812/T80 mixtures in distilled water at 25°C.

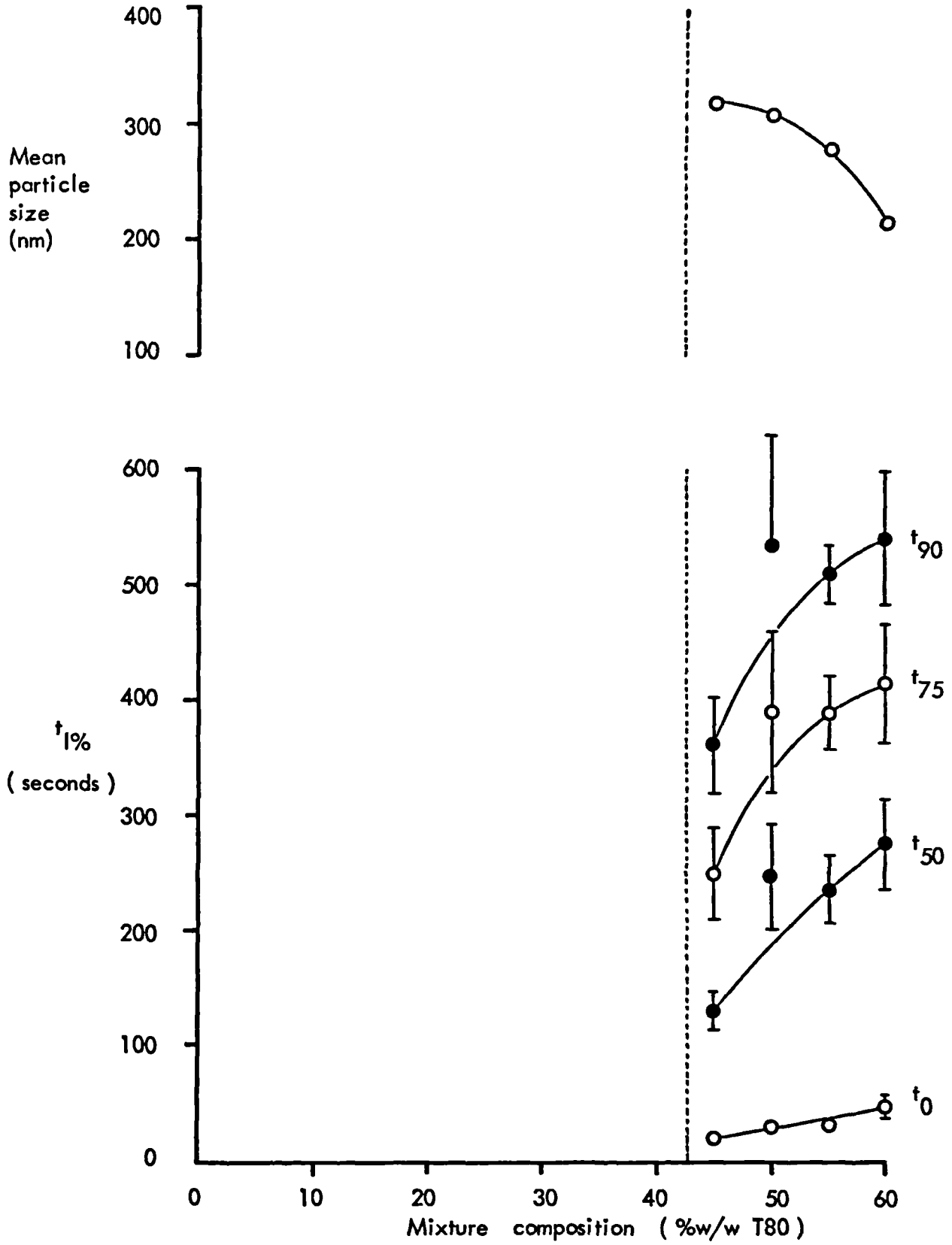
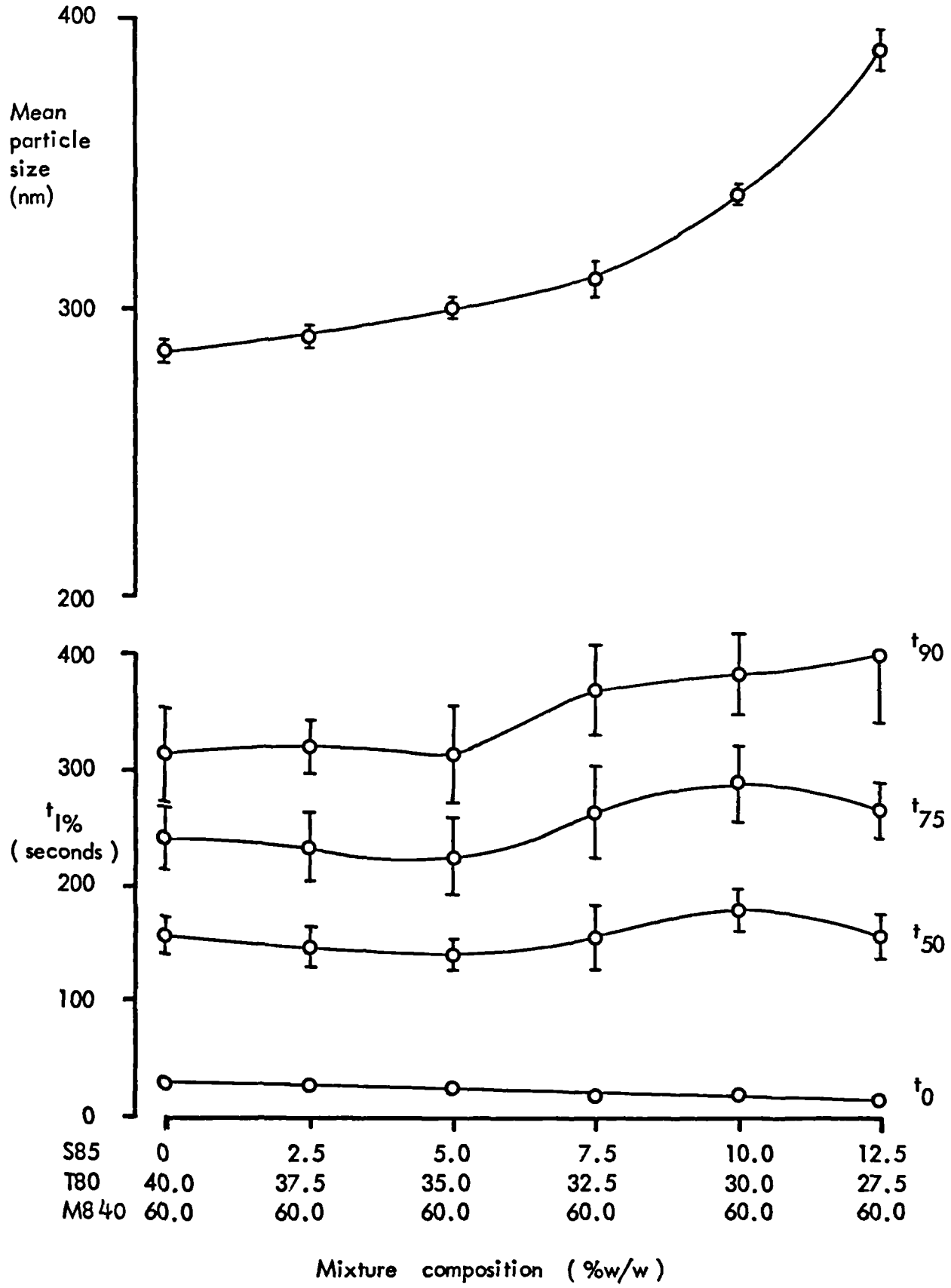


Figure 4.41 Self-emulsification of some M840/T80/S85 mixtures in distilled water at 25°C.



(see section 2.2.) Emulsification due to surfactant diffusion would also explain why the process was comparatively slow and why with increasing T80 concentration the emulsions became progressively finer as the systems became more solubilized. Mixtures containing greater than 80%T80 were extremely slowly emulsifying but eventually lead to fine emulsions just as 100%T80 eventually dissolved to form a micellar solution. Using this theory the effect of S85 (Figure 4.41) could be explained by envisaging that the S85 acted as an oil. Thus, the effect was similar to a decrease in T80 concentration; the particle size increased. The fact that the $t_{I\%}$ values did not fall with inclusion of S85 might be explained by the greater affinity of S85 (with respect to Miglyols) for T80, resulting in a slower diffusion rate.

4.4. THE EFFECT OF CONTINUOUS PHASE COMPOSITION ON SELF-EMULSIFICATION OF M812/T85/S85 MIXTURES

Dissolved substances in the aqueous continuous phase may have profound effects on surfactants and their emulgent properties. The gastric contents may contain a wide variety of dissolved substances and therefore it was necessary to consider how self-emulsifying mixtures given by mouth might be affected by the gut environment. Some materials may enhance emulsification or emulsion stability. These are generally materials which could loosely be described as co-solvents; long chain alcohols typify this group. These are not present in normal gastric contents although bile salts may have similar properties. The emphasis of this section is on electrolytes, which will certainly be present in the stomach but unfortunately tend to have deleterious effects on the integrity of emulsions.

The well-documented effects of electrolytes on ionic surfactant systems may be dramatic. Micellization is generally enhanced due to decreased repulsion of polar head groups (see, for instance, ref. 162 chapters 1 and 2) and stability of emulsions is lowered due to modification of electrostatic repulsion between particles (see ref. 190). Electrolyte effects on non-ionic emulsion systems are generally regarded as being less dramatic although they certainly do occur. The stability of non-ionic systems is often retained in the presence of electrolytes because

the stability of such systems is not reliant on electrostatic repulsion between particles[191-192]. However there are many reports in the literature concerning lowering of cloud points for aqueous surfactant solutions in the presence of electrolytes[119,193-200]. The relative effects of each ionic species are somewhat confused, not least because the effects have been compared in different ways; by way of %w/w, molarity and ionic strengths of solutions. Generalising, most electrolytes depressed cloud point over the range 0.1-1.0 Molar and higher valency anions such as sodium silicate or carbonate had greater effects than sodium or calcium chloride. The electrolyte effects on non-ionic surfactants may be due to collapse of the polyoxyethylene coil conformation[108].

The experiments concerned with electrolyte effects on self-emulsification fall into two sections. Firstly, the effect of a series of electrolytes (HCl, NaCl, $CaCl_2$, NaOH and sodium polyphosphate) on the emulsification of the model mixture (70%M812/30%T85). Secondly, an examination of the effects of HCl (which was of particular interest due to its presence in gastric secretions) on a series of M812/T85/S85 mixtures. The self-emulsification of these mixtures in distilled water has been discussed in section 4.1.3.2.

Table 4.7. shows the effects of various concentrations of hydrochloric acid on self-emulsification of the model mixture. Concentrations of less than 10^{-4}M HCl had little or no effect on emulsification rate or quality of the resultant dispersion. As HCl concentration increased to 10^{-3}M emulsification times lengthened sharply (t_{90} values increased five-fold) and there was a corresponding deterioration in emulsion quality. Between 10^{-3}M and 10^{-1}M the emulsification times did not change as significantly but showed a broad peak followed by a gradual decrease for HCl concentrations above 10^{-2}M. Particle size data obtained using the Nanosizer shows that emulsions became coarser up to 10^{-2}M HCl and then appeared to become finer between 10^{-2} and 10^{-1}M HCl. Microscopical examination of the emulsions revealed that the size range widened as HCl concentration increased; conspicuously between 10^{-2} and 10^{-1}M HCl. Emulsions formed in 10^{-1}M HCl were widely dispersed with some very large particles. In other terms, in 10^{-1}M HCl the mixture had Type A behaviour. One might say that the transition from Type B to Type A occurred at approximately 10^{-2}M HCl. After this transition had taken place the use of the Nanosizer became inappropriate for evaluation of emulsion quality. Mean particle sizes were lowered but standard deviations and polydispersity indices rose. In addition the use of emulsification times as a comparison between emulsification rates became misleading.

Table 4.7. Self-emulsification of 70%M812/30%T85 in hydrochloric acid solution at 25°C.

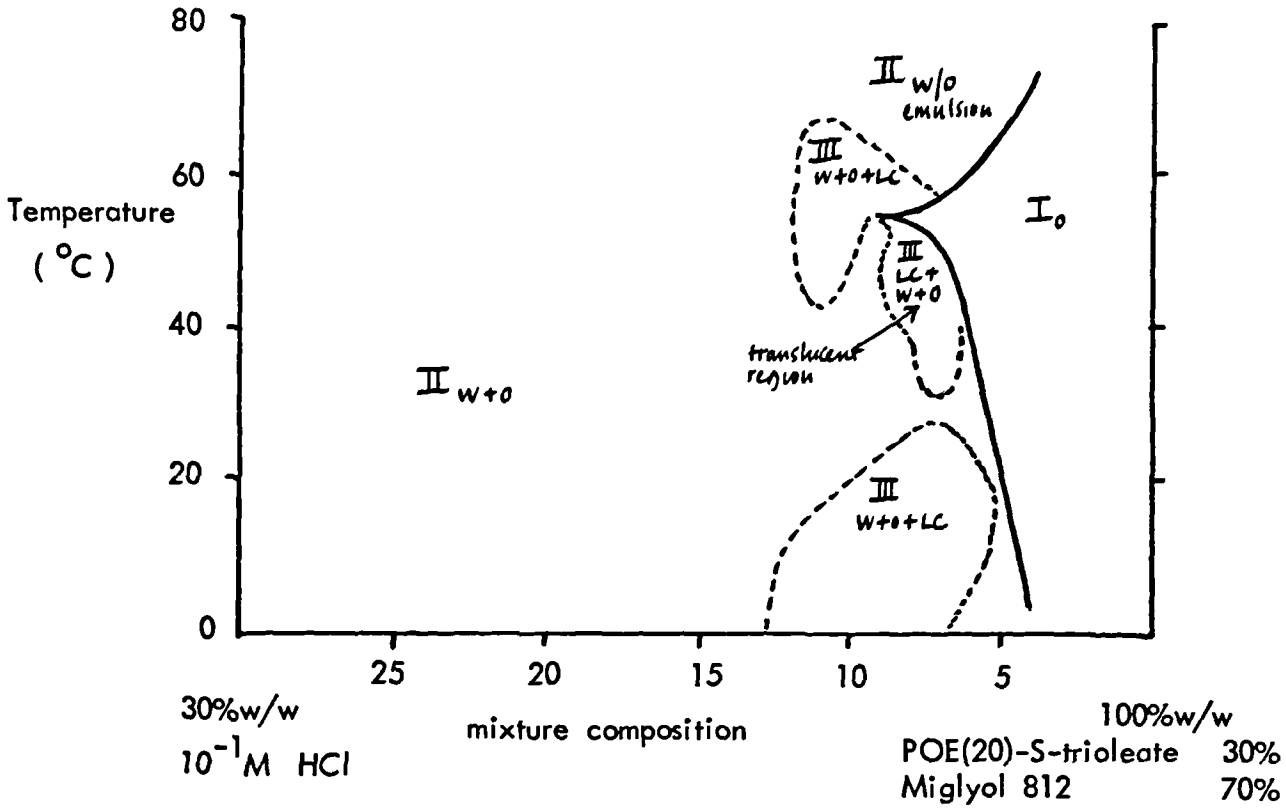
(mean and standard deviation of five replicates)

Molar HCl concn.	REL. I_{100}	$t_{1\%}$ (seconds) t_0	t_{50}	t_{75}	t_{90}	D_{0-50} ($sec.\%^{-1}$)	Mean particle size (nm)
distilled water	81.7 3.7	3.2 0.3	6.5 0.4	8.5 0.8	11.3 1.4	0.065 0.009	264 3
10^{-4}	85.0 2.1	3.3 0.2	6.6 0.5	8.3 1.0	11.1 3.3	0.063 0.012	266 4
2×10^{-4}	81.8 1.8	4.0 0.2	8.4 0.3	11.6 1.0	20.1 1.8	0.088 0.006	270 7
4×10^{-4}	77.8 3.4	4.0 0.2	10.9 0.8	18.6 1.7	30.3 3.7	0.138 0.016	-
5×10^{-4}	75.8 3.3	3.9 0.4	14.1 1.5	24.6 3.4	40.0 5.4	0.204 0.028	282 6
10^{-3}	82.6 2.2	5.2 0.2	20.0 1.2	33.6 3.8	58.8 6.4	0.296 0.027	312 6
5×10^{-3}	87.3 2.1	4.5 0.0	23.8 0.6	37.8 1.0	55.5 1.9	0.387 0.012	360 8
10^{-2}	96.8 2.5	4.5 0.9	21.2 2.2	33.9 3.2	53.2 5.6	0.334 0.043	397 7
5×10^{-2}	87.4 3.5	4.7 0.4	22.1 1.5	34.8 2.6	51.2 3.2	0.348 0.032	356 17
10^{-1}	85.0 0.3	4.8 0.4	18.9 1.0	27.6 1.3	42.3 2.6	0.283 0.026	343 16

The phase behaviour of the model mixture and 10^{-1}M HCl (Figure 4.42) was certainly different from the corresponding phase behaviour in distilled water (Figure 4.14). There was no obvious change in the critical solubilization temperature. (Other authors have described lowering of the critical temperatures, cloud point and PIT, but large changes were only observed at higher ion concentrations). However the liquid crystal formation in the current study appeared to be supressed in the presence of 10^{-1}M HCl. Birefringence was weak in all of the regions in which liquid crystals formed in corresponding distilled water systems. In many mixtures containing 10^{-1}M HCl no liquid crystal was detected. The regions of liquid crystal formation were vague and are shown contained within the dotted lines drawn in Figure 4.42. It seems that in 10^{-1}M HCl the initial penetration by the aqueous phase at 25°C resulted in formation of two phases; typical phase behaviour for Type A mixtures (See section 4.1).

The effects of all the other ionic species on self-emulsification were similar to the effects of HCl. All resulted in slower self-emulsification, coarser emulsions and transition to Type A behaviour at high concentrations of electrolytes. The relative potencies for the simple materials were HCl > NaOH > $CaCl_2$ > NaCl if compared by Molar concentration. (Calgon had a greater effect than HCl if an average molecule was considered to be that described in Chapter 3).

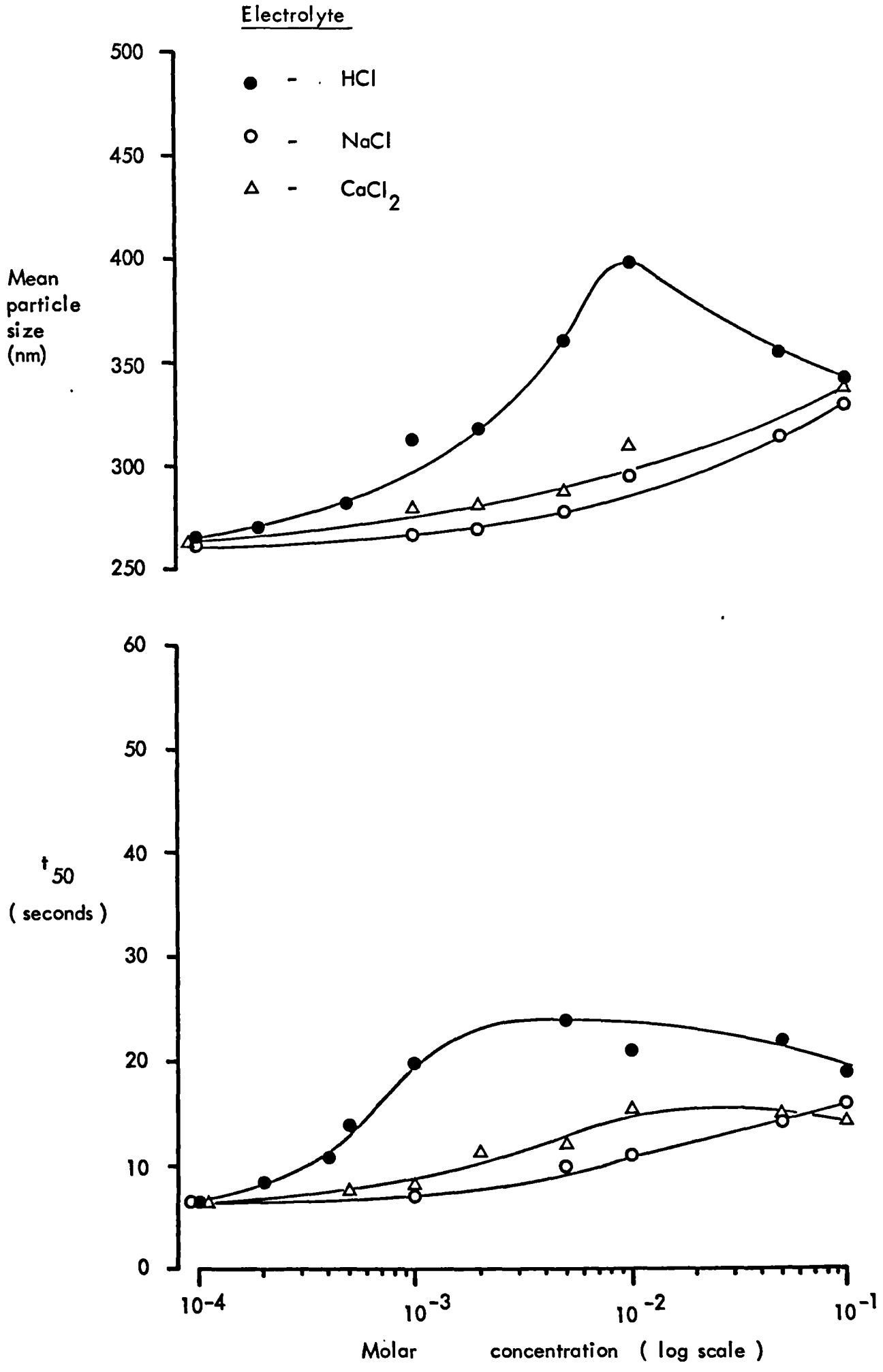
Figure 4.42 Phase diagram of 10^{-1}M HCl and the mixture 30% POE(20)-S-trioleate and 70%M812.



The relative effects of HCl, $CaCl_2$ and NaCl are indicated by Figure 4.43. In all cases the changes in $t_{50\%}$ corresponded to changes in particle size. It can be seen from the diagram that the transition to Type A behaviour for the mixture in $CaCl_2$ and NaCl solutions did not take place until electrolyte concentration was above 10^{-1}M. Type A behaviour was obvious in molar electrolyte solutions.

The effect of HCl on self-emulsification was not particular to the 70%M812/30%T85 mixture. A series of M812/T85/S85 mixtures behaved in a similar fashion. Plates 15,16 and 17 are photomicrographs of emulsions formed by the mixture 70%M812/25%T85/5%S85 in distilled water, 10^{-3}M HCl and 10^{-1}M HCl respectively. Comparing Plates 15 and 16 it can be seen that in the presence of 10^{-3}M HCl there was a large increase in the number of particles which were resolved by light microscopy. This indicates the higher mean particle size of the emulsion formed in 10^{-3}M HCl. The emulsion formed in 10^{-1}M HCl (Plate 17) included some very large particles typical of Type A emulsification. For mixtures containing S85 the detrimental effects of S85 on self-emulsification were additional to the effects caused by the presence of HCl. Therefore the $t_{50\%}$ values increased as HCl concentration increased and also as S85 concentration in the mixture increased, as long as behaviour remained Type B (Table 4.8.). Corresponding changes of particle sizes of resultant emulsions were observed, again, up to the point before behaviour became Type A (Table 4.9). The transition point

Figure 4.43 Self-emulsification of 70%M812/30%T85 in the presence of ions at 25°C.



Plates 15-17 The effect of hydrochloric acid dissolved within the aqueous phase on quality of emulsions formed by self-emulsification of 70%M812/25%T85/5%S85 at 25°C.

100μm

Plate 15 Distilled water

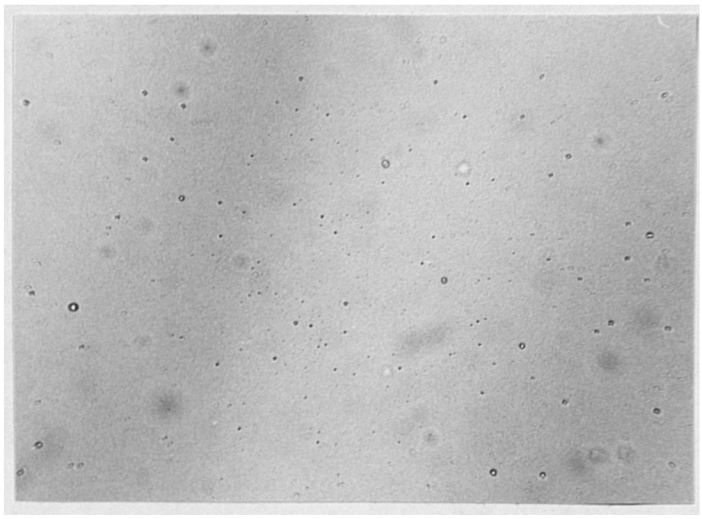


Plate 16 10^{-3}M HCl

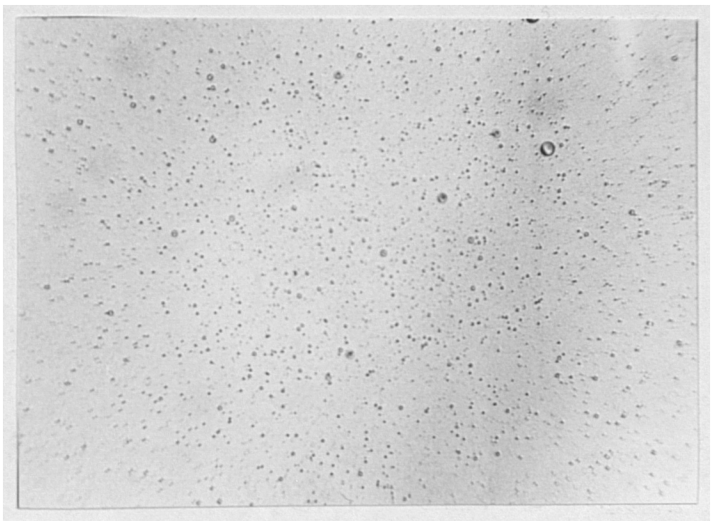
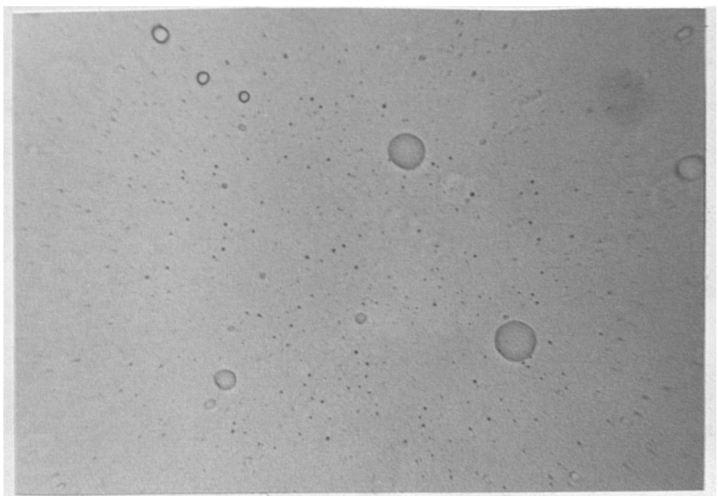


Plate 17 10^{-1}M HCl



between Type B and Type A behaviour could be detected by reference to photomicrographs of the emulsions and this transition is indicated by the dotted lines on each Table. Behaviour to the top-left of the dotted lines was Type B. In contrast behaviour to the botton-right of each line was Type A. Table 4.9 shows clearly that Type A mixtures had higher standard deviations of particle size and higher polydispersity indices, both indications of poor emulsion quality. Graphical presentation of the data plotted against hydrochloric acid concentration (Figure 4.44) or against mixture composition (Figure 4.45) show that trends in both measurements were general for Type B emulsification. After the transition to Type A behaviour values of $t_{I\%}$ were short and the particle size data became misleading. It was interesting to note that whilst there was little difference in $t_{50\%}$ for mixtures with added S85 when emulsified in distilled water (Figure 4.44), the additional presence of HCl resulted in expression of higher t_{50} values. The self-emulsification of mixtures in which S85 had been included in place of part of the M812 (also tabulated in Tables 4.8 and 4.9) was considerably worse than that of mixtures in which S85 had replaced some T85. The position of the Type B to Type A transition boundaries show that in the former case far less S85 could be tolerated. This emphasises the importance of the nature of the oil and its concentration for self-emulsification.

Table 4.8 Emulsification times ($t_{50\%}$ values) of M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C.

MIXTURE COMPOSITION %w/w			MEAN t_{50} VALUES AND S.D. (SECONDS) Molar hydrochloric acid concentration						
M812	T85	S85	10^{-4}M	2 x 10^{-4}	5 x 10^{-4}	10^{-3}	5 x 10^{-3}	10^{-2}	10^{-1}
70	30	-	6.6 0.5	8.4 0.3	14.1 1.4	20.0 1.2	23.8 0.6	21.2 2.2	18.9 1.0
70	27.5	2.5	6.9 0.4	8.7 0.8	14.5 1.6	25.4 2.2	32.2 1.4	33.7 3.0	23.9 3.5
70	25	5	7.0 0.5	9.0 0.6	14.4 0.9	30.5 2.7	43.6 5.8	47.1 3.5	25.5 2.0
70	22.5	7.5	7.5 0.5	9.0 0.7	13.3 1.0	30.9 2.4	41.4 3.5	34.8 5.9	26.6 2.4
70	20	10	5.2 0.3	6.4 0.2	9.4 0.5	33.5 3.4	-	19.8 2.1	14.2 1.0
67.5	30	2.5	6.9 0.4	8.4 0.4	14.8 0.9	23.5 2.7	-	43.8 2.9	23.0 2.3
65	30	5	6.8 0.4	9.0 0.5	14.1 2.1	56.0 7.9	-	78 6	39 2
62.5	30	7.5	6.0 0.4	7.1 0.3	14.5 2.4	29.0 2.0	-	53 12	19 2
60	30	10	6.3 0.3	6.8 0.7	15.5 1.2	- -	-	17 1	27 2

Table 4.9 Quality of emulsions formed by self-emulsification of M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C.

MIXTURE COMPOSITION %w/w			MEAN PARTICLE SIZE (nm), S.D. AND P.I. Molar hydrochloric acid concentration				
M812	T85	S85		distilled water	2×10^{-4}	10^{-3}	10^{-2}	10^{-1}
70	30	-	mean	264	270	312	397	343
			s.d.	3	7	6	7	16
			P.I.	1	1	3	3	3
70	27.5	2.5	mean	274	285	311	411	370
			s.d.	5	5	2	11	14
			P.I.	2	3	3	4	5
70	25	5	mean	316	327	388	441	429
			s.d.	2	8	6	10	13
			P.I.	3	3	4	5	4
70	22.5	7.5	mean	346	391	412	469	424
			s.d.	4	4	14	13	4
			P.I.	3	4	4	6	5
70	20	10	mean	322	389	386	333	289
			s.d.	4	26	17	6	5
			P.I.	5	5	5	3	5
67.5	30	2.5	mean	277	305	337	410	352
			s.d.	8	9	7	10	5
			P.I.	2	3	4	5	5
65	30	5	mean	293	307	297	349	320
			s.d.	8	13	11	2	6
			P.I.	4	4	5	4	4
62.5	30	7.5	mean	289	289	330	329	315
			s.d.	7	7	5	9	3
			P.I.	4	4	5	5	5
60	30	10	mean	290	291	320	329	322
			s.d.	11	8	16	31	25
			P.I.	5	5	5	5	6

Figure 4.44 Self-emulsification of some M812/T85/S85 mixtures in hydrochloric acid solution at 25°C.

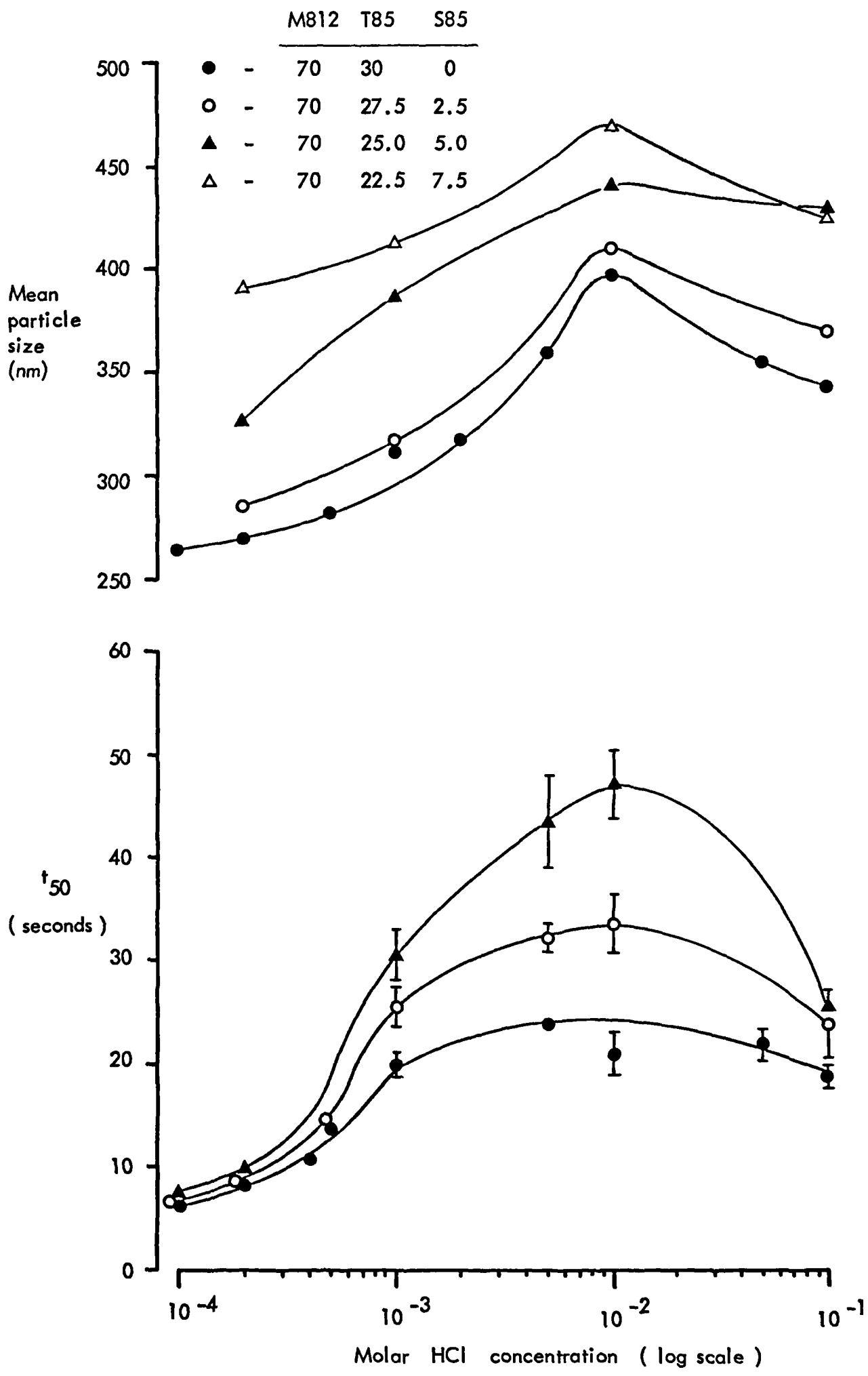
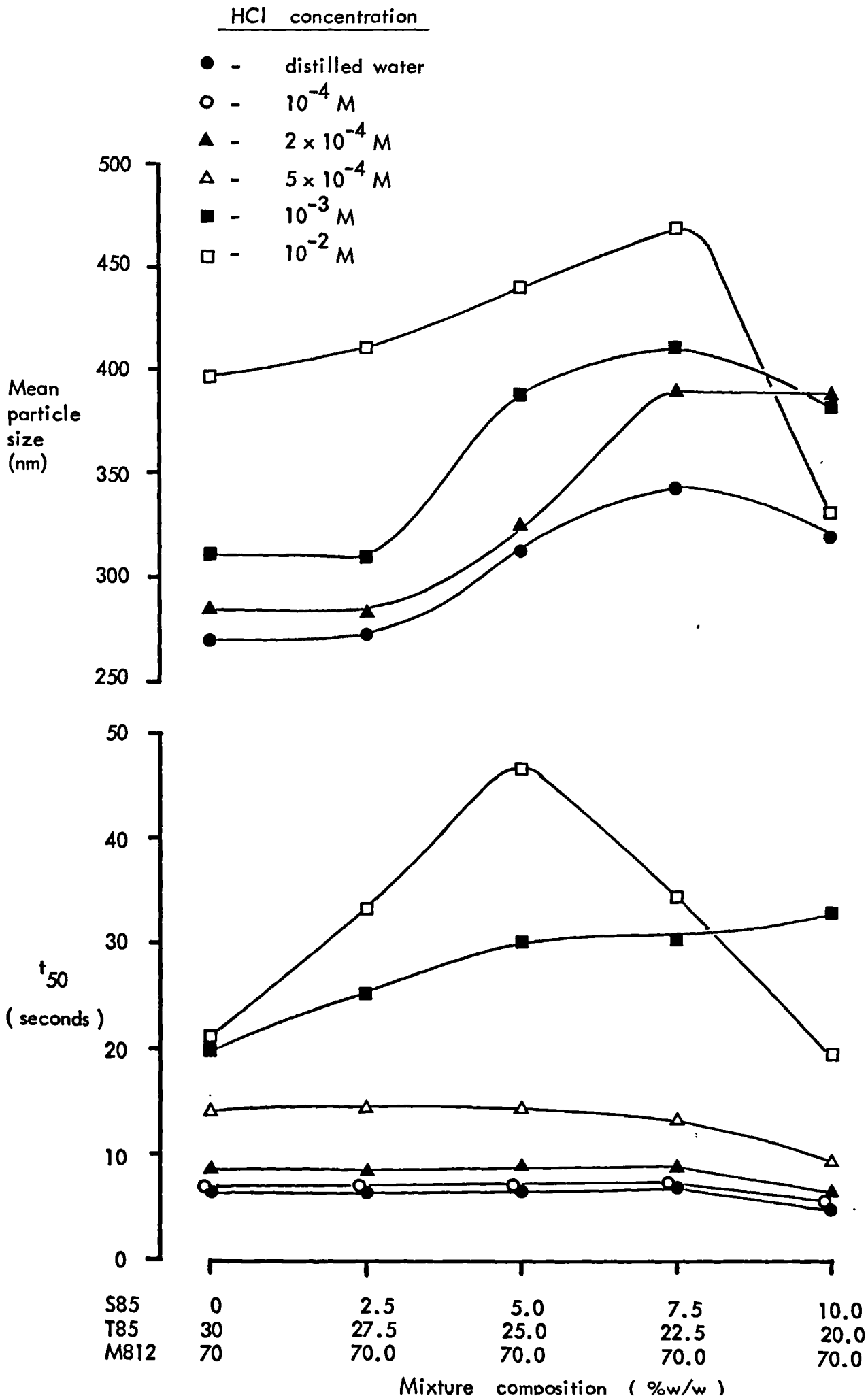


Figure 4.45 Self-emulsification of some M812/T85/S85 mixtures in hydrochloric acid solutions at 25°C.



The particle sizes of homogenised emulsions of the model mixture were not affected until HCl concentration was higher than 10^{-2}M. Even in 10^{-1} HCl the particle size was only slightly raised (Table 4.10). Similarly there was little variation in particle size of homogenised dispersions of T85 in HCl solutions. Although, on visual inspection the T85 dispersions differed in turbidity. By measuring relative turbidity (as described in Chapter 3) it was possible to compare the affinities of T85 for various electrolyte solutions. Table 4.11 lists relative slopes of intensity of light scattering versus concentration of T85. A linear relationship held over the concentration range (10^{-3} to 10^{-1}%w/w T85) for each electrolyte concentration. The marked change in relative turbidity occurred between 10^{-4}M and 10^{-3}M HCl which corresponded to the change in emulsification properties above 10^{-4}M HCl. This suggests that the effects of ionic species on self-emulsification were due to a change in T85/H_2O interaction which is manifest in the phase diagrams (Figure 4.42). The turbidity of T85 dispersions was also raised by the other electrolytes as shown in Table 4.11.

The current study shows that emulgent properties of non-ionic surfactants are adversely affected by low concentrations of electrolytes as well as the higher concentrations investigated previously[119, 193-200].

Table 4.10 Effect of hydrochloric acid concentration on particle size of homogenised emulsions of 70%M812/30%T85 at 25°C.

MOLAR HYDROCHLORIC ACID CONCN.	PARTICLE SIZE (nm) Mean	S.D.	P.I.
distilled water	189	1	2
10^{-3}M	187	4	3
10^{-2}M	189	5	3
10^{-1}M	200	3	3

Table 4.11 Light scattering of homogenised T85 dispersions (of the range 10^{-3} to 10^{-1} %w/w) in the presence of ions.

Molar concentration and identity of ionic species	Linear regression analysis Rel ($I - I_o$) versus T85 concn.	
	Relative slope	correlation coefficient
distilled water	4.8	0.9998
10^{-4}M HCl	5.0	0.9999
2×10^{-4}M HCl	8.1	0.9996
5×10^{-4}M HCl	13.0	0.9994
10^{-3}M HCl	13.8	0,9969
10^{-2}M HCl	14.0	0.9997
10^{-1}M HCl	15.0	0.9995
10^{-1}M NaCl	14.0	0.9997
10^{-1}M $CaCl_2$	16.2	0.9988

4.5. THE EFFECT OF BENZOIC ACID DISSOLVED WITHIN M812/T85 MIXTURES ON SELF-EMULSIFICATION

The self-emulsification of M812/T85/benzoic acid mixtures was examined primarily because such a mixture was to be used as a biological model. In addition, there was a general interest in how dissolved drugs might affect self-emulsification. Infact, the effects of dissolved benzoic acid were more interesting than had been anticipated.

Various concentrations of benzoic acid in the model mixture were studied. Data is represented graphically in Figures 4.46 and 4.47. At low concentrations of benzoic acid (below 0.2%) $t_{50\%}$ became longer and particle size increased for self-emulsification in distilled water. Above 0.2% benzoic acid the trend reversed and as concentration of benzoic acid was increased above 1% the emulsification became more efficient than that of 0% benzoic acid mixtures. Particle size was lowest at approximately 2% benzoic acid. Further increase in benzoic acid concentration towards saturation resulted in higher particle sizes, although these were still much lower than that of the 0% benzoic acid mixture. The t_{50} between 2% and 7.5% benzoic acid shortened steadily although these changes were not as great as the changes in particle size.

Figure 4.46 Effect of solubilized benzoic acid on self-emulsification of 70%M812/30%T85 at 25°C.

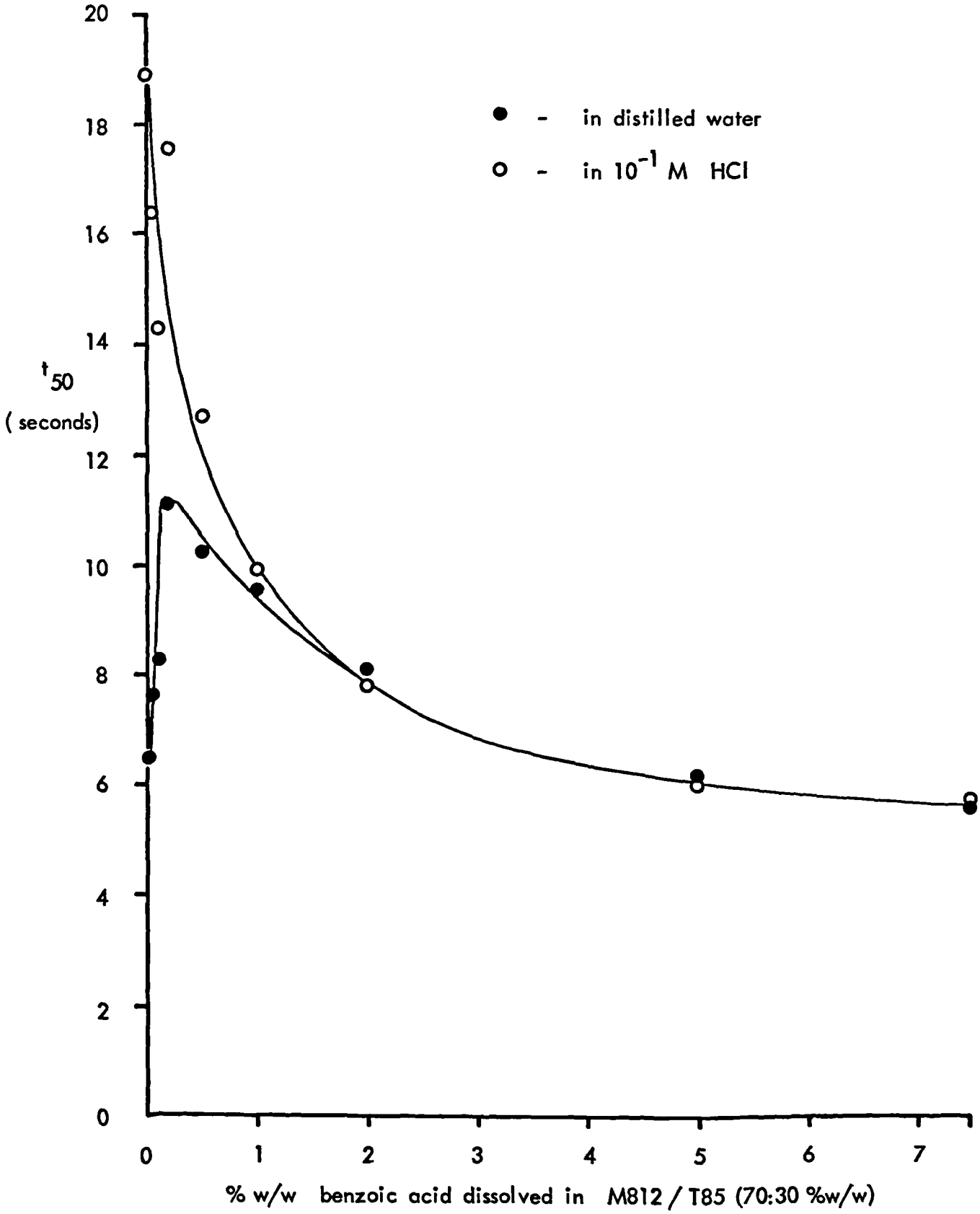
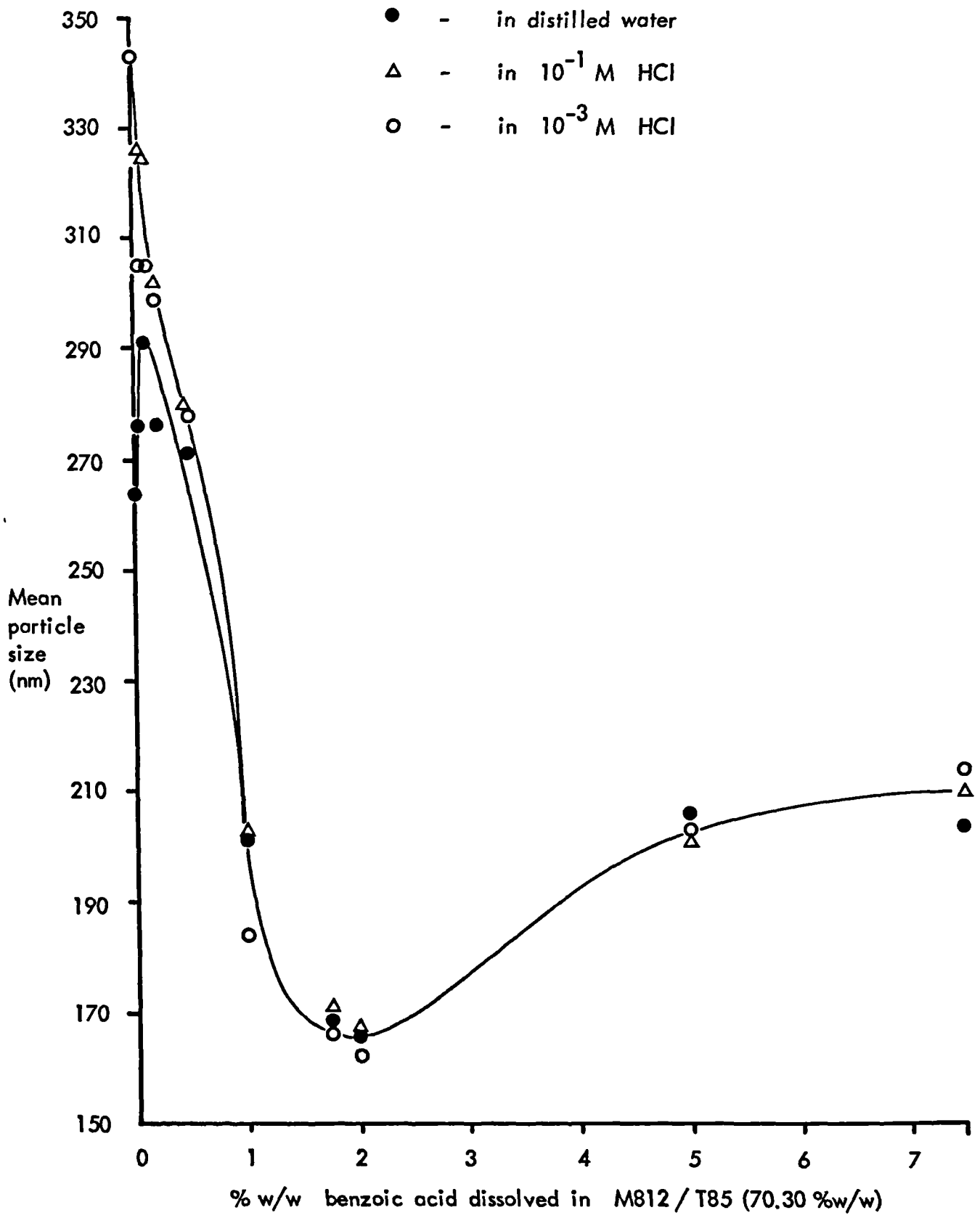


Figure 4.47 Effect of solubilized benzoic acid on mean particle size of emulsions formed by self-emulsification at 25°C.

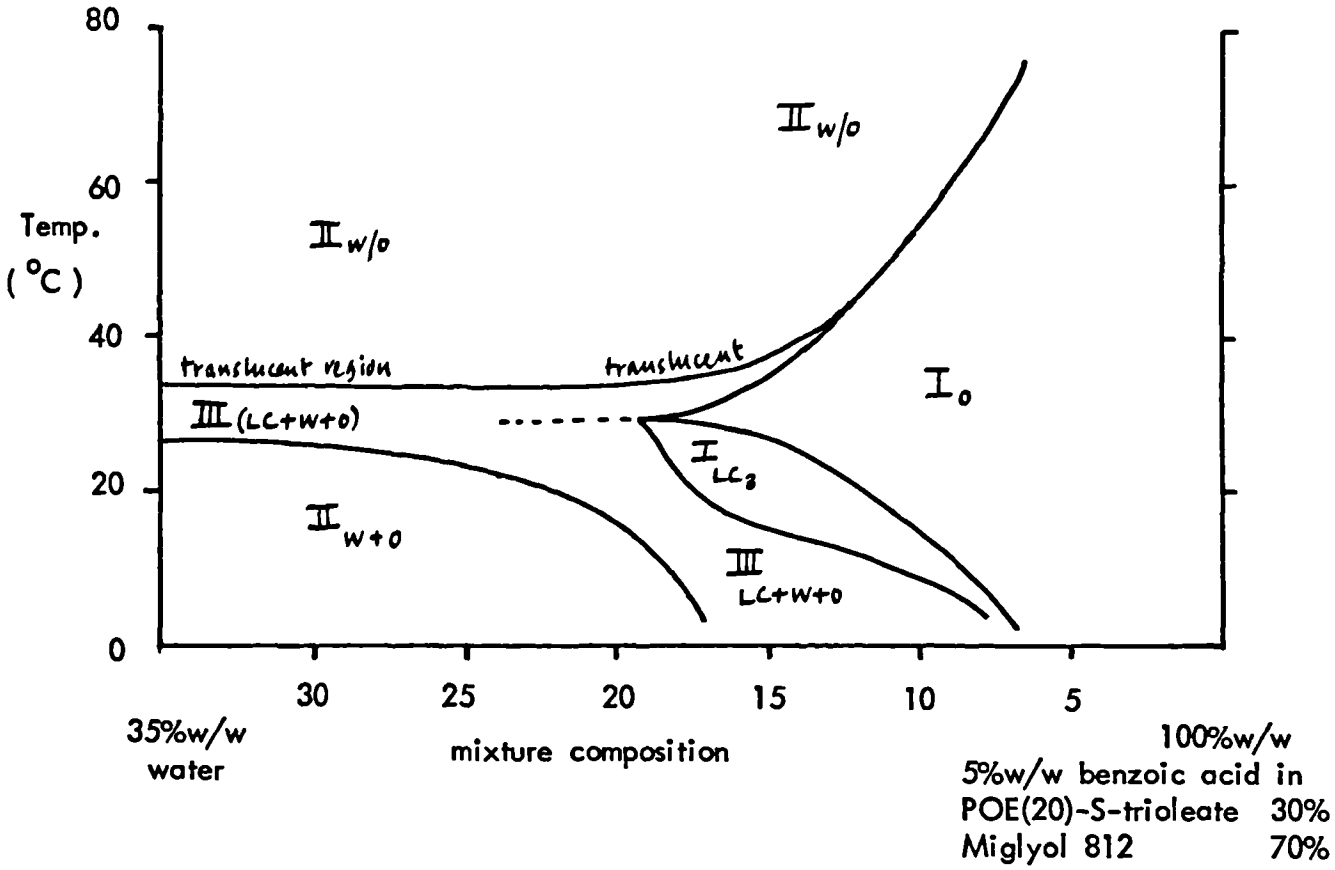


Crucially, the deleterious effects of HCl on self-emulsification were overcome by the presence of benzoic acid. The $t_{50\%}$s and particle sizes for mixtures with benzoic acid concentrations greater than 1%^{w}/w were unaffected by HCl concentrations up to 10^{-1}M. Therefore benzoic acid would aid emulsification in the gastric contents.

A 5% benzoic acid mixture was to be used for the biological study. Therefore the properties of this mixture were examined in more detail. The phase behaviour of the 5% benzoic acid mixture and water (Figure 4.48) explained the occurence of efficient self-emulsification. The CST was lowered to about 30°C and the I_o region was larger than that in Figure 4.14. The liquid crystalline region I_{LC3}, which seemed to be an important indicator of efficient self-emulsification, was large. The translucent, phase inversion region (III_{LC+w+o}) contained large quantities of birefringent material and extented to high water contents. Thus, emulsification of the 5% benzoic acid mixture at 25°C was optimised by the low interfacial tensions close to the CST and, possibly, by the readily-formed liquid crystalline phase.

The nature of the I_o phase was similar to that for 0% benzoic mixtures although larger quantities of water could be solubilized by the 5% benzoic acid mixture at 25°C. Solubilized water had similar effects on density and viscosity of the original I_o mixtures. Densities increased

Figure 4.48 Phase diagram of water and the mixture 5% benzoic acid in 30%POE(20)-S-trioleate/ 70%M812.



non-linearly with increasing water content and the viscosities increased to approximately the same maximum as that of 0% benzoic acid mixtures before the phase transition to liquid crystal (Figure 4.49). Emulsification time was not affected by moisture content until the phase transition (Table 4.12) and whilst dispersion of the I_{LC3} phase was slower (16.27% H_2O) the result was an emulsion of comparable quality (Table 4.13). This behaviour was analogous to the behaviour of 0% benzoic acid mixtures (section 4.1.3.3). Quality of resultant emulsion was not lost until the mixture to be self-emulsified became II_{w+o} type (Table 4.13).

Clearly the chemical nature and concentration of a drug solubilized within a self-emulsifying mixture may affect the efficiency of self-emulsification. The governing factors are, one would suspect, concerned with the interaction of the dissolved substance with the surfactant. Perhaps a small quantity of drug dissolved in the oil without significant interaction with the surfactant molecules would not affect emulsification. In the case of benzoic acid saturated solution in the 70%M812/30%T85 mixture was 50% more concentrated.than saturated solution in M812. This indicates that the benzoic acid did interact with the T85 micelles. Other authors have reported work on solubilization of benzoates by non-ionic surfactants[201-202]. The work of Mukerjee[202] suggests that benzoic acid is partially solubilized in the polyoxyethylene mantle of

Figure 4.49 Effect of water concentration on viscosities and densities of emulsifiable mixtures in the presence of benzoic acid (at 25°C).

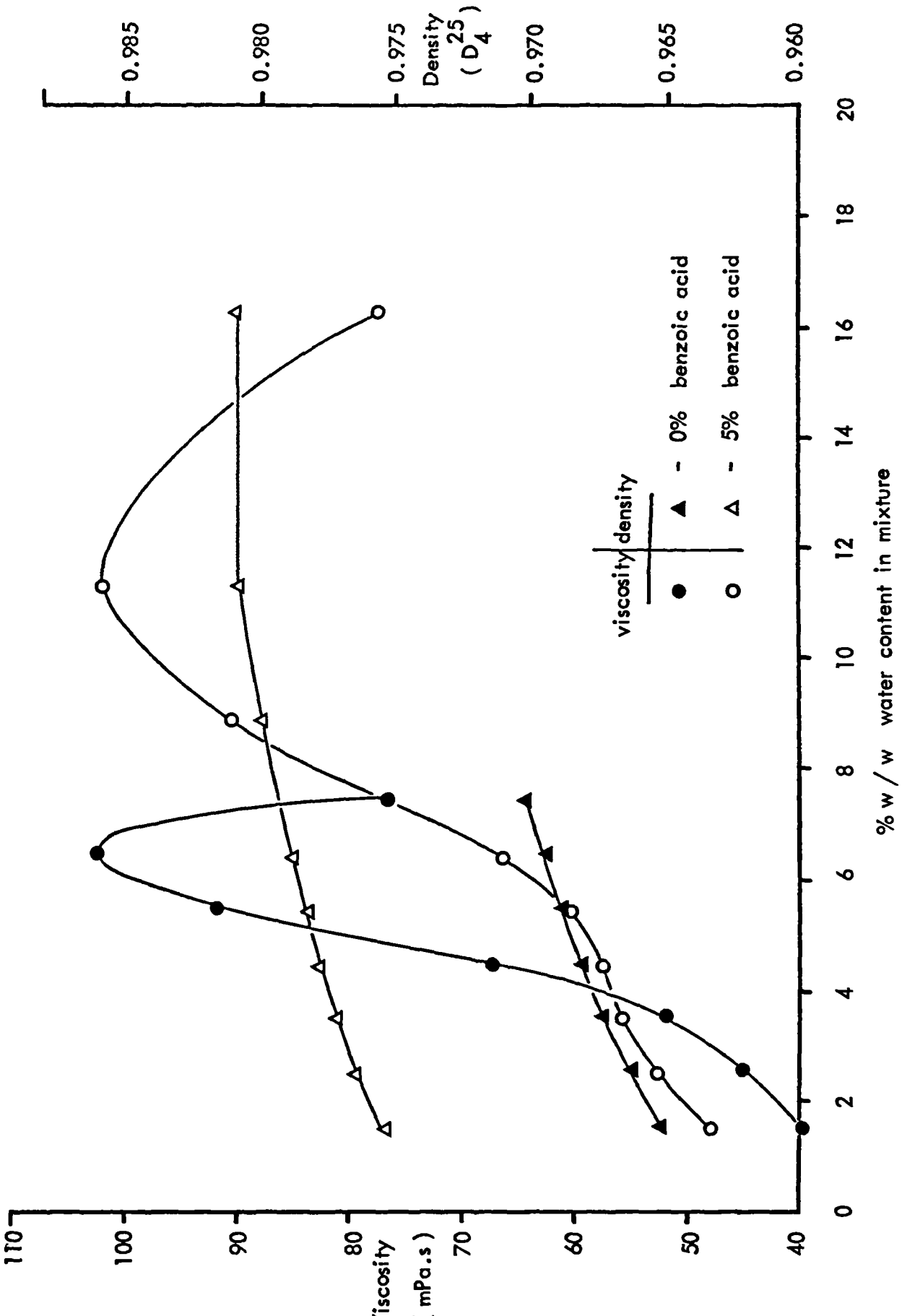


Table 4.12 Effect of water content (of original mixtures) on emulsification rate of mixtures containing benzoic acid.
($5\%^{w}/w$ benzoic acid in 70%M812/30%T85)$_o$ plus water mixtures in distilled water at 25°C.

TOTAL WATER CONTENT OF MIXTURE %w/w	MEAN $t_{1\%}$ VALUES AND S.D. (seconds)				D_{0-50} (sec.%$^{-1}$)
	t_0	t_{50}	t_{75}	t_{90}	
1.49	3.6 0.2	6.1 0.2	7.4 0.2	8.7 0.2	0.049 0.003
3.46	3.2 0.2	5.6 0.2	6.9 0.3	8.0 0.6	0.049 0.006
6.42	3.7 0.3	6.1 0.2	7.4 0.2	8.5 0.3	0.049 0.005
8.88	3.9 0.2	6.3 0.2	7.6 0.2	8.6 0.3	0.049 0.005
11.34	3.9 0.3	6.5 0.2	7.9 0.2	9.7 0.3	0.052 0.002
16.27	4.1 0.2	8.2 0.6	11.7 0.8	17.8 1.1	0.082 0.009

Table 4.13 Effect of water content (of original mixture) on quality of emulsions formed by self-emulsification of mixtures containing benzoic acid ($5\%^{w}/w$ benzoic acid in 70%M812/30%T85) plus water mixtures in distilled water at 25°C.

TOTAL WATER CONTENT OF MIXTURE %w/w	Phase characteristics of mixture at 25°C	PARTICLE SIZE (nm)		P.I.
		Mean	S.D.	
1.49	I_o	203	4	1
2.48	I_o	201	4	1
3.46	I_o	202	4	1
4.45	I_o	202	4	1
5.43	I_o	204	3	1
6.42	I_o	203	4	1
8.88	I_o	203	4	1
11.34	I_o	206	2	2
16.27	I_{LC}	206	4	2
21.19	III_{LC+o+w}	210	4	2
29.12	III_{o+w}	>3000	-	-

aqueous non-ionic micelles. Its distribution depends on its concentration. This may explain why low concentration of benzoic acid in the oil/surfactant mixture retarded dispersion and resulted in poorer emulsions.

4.6. THE IMPORTANCE OF CONDITIONS DURING EMULSIFICATION TO SUBSEQUENT EMULSION QUALITY

The stability of emulsions formed by self-emulsification was not of direct interest to the current study. However it was interesting to note that most emulsions were highly metastable. One must bear in mind though that they were dilute emulsions and these would be more stable than concentrated emulsions due to the lower frequency of particle collisions.

An estimate of the stability of emulsions was obtained by measurement of particle size after various storage times. Table 4.14 shows that there was little change in the particle sizes of self-emulsified or homogenised M840/T85 systems over a two day period. The fact that there were no obvious changes in the self-emulsified systems suggests that self-emulsification was not a continuing process. Once equilibrium had been reached, which was always within five minutes for the systems tablulated, the quality of emulsions did not improve after storage. There was little change even for high concentrations of T85. Molecular diffusion of surfactant was not significant due to the poor water solubility of T85. In contrast T80/oil systems did change slowly, due to molecular diffusion, when large proportions of T80 were present.

Table 4.14 Effect of storage on quality of emulsions formed by self-emulsification and homogenisation of M840/T85 mixtures in distilled water at 25°C.

MIXTURE COMPOSITION %w/w		MEAN PARTICLE SIZE (nm), S.D. AND P.I.				
			Self-emulsified systems		Homogenised systems	
M840	T85		t = 1 hr	t = 2 days	t = 1 hr	t = 2 days
85	15	mean	211	235	278	284
		s.d.	12	6	13	7
		P.I.	5	3	4	4
80	20	mean	283	285	226	225
		s.d.	16	5	1	1
		P.I.	2	2	3	2
75	25	mean	276	274	211	212
		s.d.	5	10	4	2
		P.I.	2	2	2	2
70	30	mean	307	294	180	181
		s.d.	7	6	2	1
		P.I.	3	1	3	3
65	35	mean	339	322	173	171
		s.d.	12	11	2	5
		P.I.	4	4	2	3
60	40	mean	393	351	191	189
		s.d.	25	47	10	4
		P.I.	7	6	2	3
55	45	mean	498	462	187	186
		s.d.	38	24	6	3
		P.I.	8	8	3	3
50	50	mean	691	588	189	184
		s.d.	123	74	7	4
		P.I.	9	9	3	3
40	60	mean	683	691	181	180
		s.d.	121	94	5	2
		P.I.	9	9	4	4

The environmental conditions during emulsification were of vital importance to self-emulsified systems. Once an emulsion had been formed and stabilised the temperature could be varied without greatly changing emulsion quality. Table 4.15 illustrates this point. As discussed previously (in section 4.1.3.4) the temperature of emulsification for the model mixture had critical effects on emulsion quality. However, once formed at a particular temperature, the emulsions could all be stored at one temperature, 25°C, without significant change in the mean particle sizes. These results confirm the usefulness of the 'emulsification-by-PIT' method which has been discussed by Shinoda[118,203] and Friberg[128]. Friberg has found that emulsification is efficient at or just below the PIT. However it seems that the emulsification is very dependant on the presence of 'surfactant phase' which may emulsify spontaneously. The current study seems to support this view. Other authors have reported no advantage in emulsification at the PIT for certain systems[129].

Table 4.15 Effect of storage at 25°C on quality of emulsions formed by self-emulsification of 70%M812/30%T85 at various temperatures (in distilled water).

Emulsification Temp. °C	Emulsion quality t = 1hr Particle size mean	S.D.	P.I	Particle size of emulsions after storage at 25°C (nm) t = 2hrs. mean	S.D.	t = 3 days mean	S.D.	t = 10 days mean	S.D.
10	368	4	3	375	3	378	9	376	5
15	365	4	2	364	5	368	4	366	6
20	307	4	1	315	2	310	2	317	1
25	269	3	1	-	-	275	0	-	-
30	230	2	1	239	4	232	4	231	2
35	208	9	0	217	4	214	2	214	2
40	213	5	1	221	1	221	4	224	4
50	250	6	2	251	4	245	2	257	6

4.7. BIOLOGICAL ABSORPTION OF BENZOIC ACID FROM FORMULATIONS ADMINISTERED ORALLY

Excretion of unmetabolised benzoic acid was not detected in any of the assays of urine contents following administration of 250mg benzoic acid. This was expected (see section 1.4). Normal excretion of hippuric acid was reasonably low providing that subjects complied with the dietary restrictions described in Chapter 3. Moreover the normal excretion of hippuric acid was quite constant. This enabled subtraction of the hippuric acid due to normal metabolism from the total, leaving the hippuric acid due to benzoic acid administration. The subtraction was performed graphically by calculating the mean rates of appearance of hippuric acid between samples (Figure 4.51). Subsequently the cumulative appearance of hippuric acid by weight due to benzoic acid administration was calculated. Excretion rates of hippuric acid could not be determined from the data due to the presence of hippuric acid from normal metabolism. Therefore it was not possible to calculate absorption rates of benzoic acid from the data. However initial parts of the curves are representative of the absorption process since excretion was rapid as reported by other authors (see 1.4).

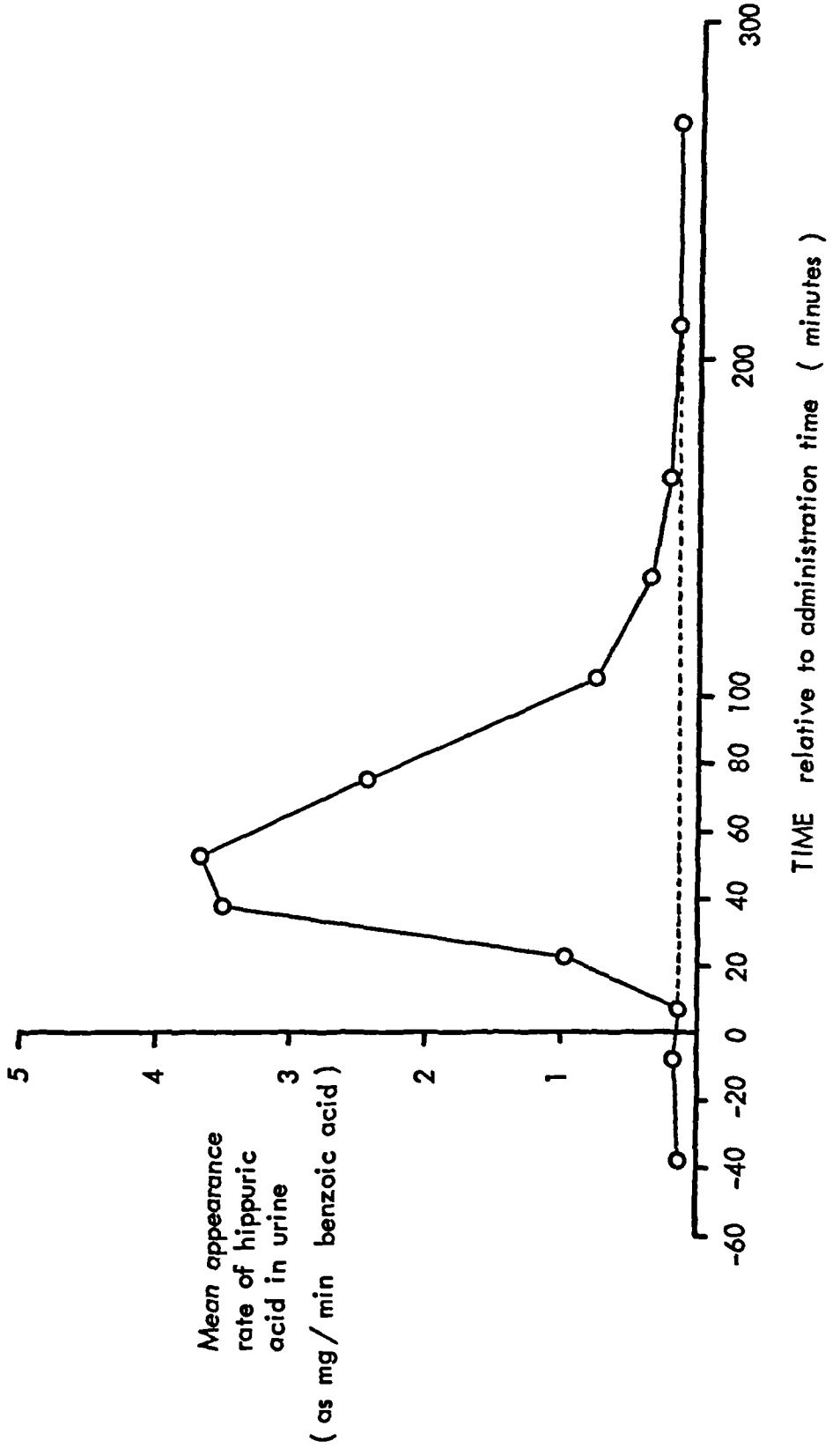
Cumulative appearance of hippuric acid (as wt. benzoic acid) is tabulated for each experiment, grouped as the various formulations (Table 4.16 to 4.20). The mean cumulative weights of hippuric acid excreted were calculated for each sample time. It appeared that the self-emulsifying formulation was excreted (and therefore absorbed) more quickly and consistently than the other

formulations. However this difference was not statistically significant as shown by Table 4.21. Table 4.21 lists the times taken for excretion of the equivalent of 125mg benzoic acid (ET_{50}) as a representative parameter from each curve. An analysis of variance was performed on the data for the three formulations administered to all six subjects. The ANOVA table shows that all the curves are likely to belong to the same population.

In summary, no significant differences between formulations were indicated by the available data. A larger number of subjects would have been desirable and may have demonstrated significant differences, although the absorption of benzoic acid was rapid for each formulation.

It is likely that absorption of benzoic acid would not be improved by formulation in a self-emulsifying vehicle.

Figure 4.50 Typical experimental plot of appearance of hippuric acid in urine following oral administration of 250mg benzoic acid.



Tables 4.16-4.20 Cumulative appearance of hippuric acid in urine resulting from oral administration of benzoic acid in various formulations.

Table 4.16 Formulation 1
powdered drug
250mg crystalline benzoic acid

SAMPLE TIME (mins.)	CUMULATIVE WEIGHT HIPPURIC ACID EXCRETED (as mg benzoic acid)						MEAN CUM. WT. (mg)	S.D.
	A	B	C	D	E	F		
15	0	0	1	3	0	0	1	1
30	13	44	25	98	61	30	45	28
45	51	90	64	174	149	91	103	44
60	75	126	106	205	180	143	139	44
90	108	177	158	219	209	187	176	37
120	133	206	183	230	225	208	198	33
150	158	224	196	236	234	225	212	28
180	201	235	209	240	240	235	227	16

Table 4.17 Formulation 2
non-emulsifying oil solution
5g (5%w/w benzoic acid solution in M812)

SAMPLE TIME (mins.)	CUMULATIVE WEIGHT HIPPURIC ACID EXCRETED (as mg benzoic acid)						MEAN CUM. WT. (mg)	S.D.
	A	B	C	D	E	F		
15	1	0	0	8	2	1	2	3
30	11	48	64	137	30	35	54	40
45	36	125	135	203	101	86	114	51
60	67	172	173	233	153	133	155	50
90	147	214	208	255	208	184	203	33
120	192	229	222	262	237	207	225	22
150	211	236	235	261	248	220	235	17
180	223	239	241	259	250	230	240	12

Table 4.18 Formulation 3
self-emulsifying formulation
5g (5%w/w benzoic acid in 70%M812/30%T85)

SAMPLE TIME (mins.)	CUMULATIVE WEIGHT HIPPURIC ACID EXCRETED (as mg benzoic acid)						MEAN CUM. WT. (mg)	S.D.
	A	B	C	D	E	F		
15	6	0	1	4	2	4	3	2
30	80	73	73	90	25	63	67	21
45	157	144	166	157	74	124	137	31
60	204	188	196	194	153	163	183	19
90	240	218	224	223	211	196	219	13
120	251	222	232	232	226	210	229	12
150	254	223	232	237	233	221	233	11
180	254	227	233	241	239	232	238	9

Table 4.19 Formulation 4
poorly emulsifying formulation
5g (5%^{w}/w benzoic acid in T85)

SAMPLE TIME (mins.)	CUMULATIVE WEIGHT HIPPURIC ACID EXCRETED (as mg benzoic acid)			MEAN CUM. WT. (mg)	S.D.
	D	E	F		
15	4	0	0	1	2
30	80	47	20	49	25
45	142	105	57	101	35
60	182	149	94	142	36
90	221	194	167	194	22
120	231	210	206	216	11
150	238	213	222	220	12
180	245	213	231	230	13

Table 4.20 Formulation 5
250mg benzoic acid + 5g (70%M812/30%T85)

SAMPLE TIME (mins.)	CUMULATIVE WEIGHT HIPPURIC ACID EXCRETED (as mg benzoic acid)			MEAN CUM. WT. (mg)	S.D.
	D	E	F		
15	1	0	0	0	0
30	36	14	9	20	12
45	91	67	39	66	21
60	129	121	75	108	24
90	190	192	124	169	32
120	229	212	163	201	28
150	245	219	188	217	23
180	250	224	210	228	17

Table 4.21 Analysis of variance of ET_{50} for three formulation groups using six subjects.

(ET_{50} = time for elimination of 50% of the benzoic acid administered by the oral route)

SUBJECT	VALUE OF ET_{50}, (minutes) Formulation		
	1 (crystalline)	2 (oil solution)	3 (emulsifying)
A	109	81	38
B	60	45	40
C	69	42	38
D	34	28	37
E	40	51	55
F	55	56	45

One-tailed ANOVA

Null hypothesis Ho : variance between formulations is equal to variance within formulations

Alternative hypothesis H_1 : variance between formulations is greater than variance within formulations

	sum of squares (SS)	degrees of freedom	'mean square' (MS)
between formulations	1088	2	544 - MS_b
within formulations	5383	15	359 - MS_w

Variance ratio (MS_b / MS_w) = 1.516

using the F-distribution, $F_{0.05}(2,15)$ = 3.68

Therefore Ho is accepted.

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

The emulsification of oil/non-ionic surfactant mixtures in excess aqueous phases has been studied under conditions of low agitation. An instrument was constructed which controlled the conditions of agitation and compared the rates of emulsification. The instrument was sensitive to differences in emulsification rate between closely related oil/surfactant mixtures. However the emulsification rates were of limited importance when mixtures with wide ranges of emulsification properties were compared. In the latter case it was more useful to compare the efficiencies of emulsification in terms of the particle sizes of resulting emulsions. The Coulter Nanosizer assessed mean particle size rapidly and was a non-invasive technique. However its use was limited to sizing of dispersions of very small particles within a narrow size range. When self-emulsification was very poor the particle size was large and the size range wide. Such emulsions could be characterised by microscopical methods although a qualitative estimate was sufficient for the current study. Indeed a subjective assessment of self-emulsification, such as the C.I.P.A.C. method described in Chapter 1, was sufficient to reveal regions of 'good' emulsification. The objective methods were useful to examine these regions in more detail. Generally, within regions of 'good' emulsification, it was desirable to obtain information regarding both emulsification rate and particle size rather than depending on one property.

Miglyol 812/Tween 85 (M812/T85) mixtures of various compositions were classed as one of four Types (A-D) depending on their ability to self-emulsify in distilled water at 25°C. Type B mixtures, which contained between 25% and 40%^{w}/$_{w}$T85, self-emulsified rapidly to produce fine dispersions. The lowest mean particle size detected was produced by one of these mixtures containing 30%T85. Type A (0-15%T85) and Type C (45-60%T85) mixtures emulsified poorly although each Type had different properties. Type D mixtures (70-100%T85) did not self-emulsify.

Viscosity and density of the original oil/surfactant mixtures were shown not to affect self-emulsification. In contrast the ease of emulsification correlated closely with particular features of the phase behaviour of oil/surfactant/water mixtures at equilibrium. Efficient self-emulsification for each M812/T85 mixture occurred at or just below a temperature at which enhanced water solubilization was detected by studies of phase behaviour at equilibrium. This temperature, the critical solubilization temperature (CST), was related to the phase inversion temperature (PIT) for M812/water/T85 emulsions. Generally the CST was raised by increasing the proportion of T85. The CST of the 70%M812/30%T85 mixture with water was about 45°C. Thus the latter mixture emulsified efficiently at ambient temperatures. Type A mixtures (0-15%T85) had CSTs below 25°C and emulsified poorly at 25°C. The CSTs of Type C mixtures (45%-60%T85) were higher

than 50°C and these mixtures emulsified slowly at 25°C to form coarse emulsions.

The efficiency of self-emulsification at or just below the CST was demonstrated by studying the effect of temperature on emulsification of the 70%M812/30%T85 mixture. Self-emulsification became more efficient as the temperature was raised towards the CST. Optimum efficiency occurred at 35-40°C. When the temperature was raised above the CST poor emulsions resulted.

The efficiency of self-emulsification at temperatures at or below the CST may be explained by extremely low interfacial tensions which occur in the region of phase inversion. However phase studies of M812/T85/water mixtures at equilibrium suggested that particular molecular interactions may have been equally important. Liquid crystalline phases were detected close to the critical solubilization region for Type B mixtures. These were thought to be 'inverse hexagonal' liquid crystals and may have been crucial to the self-emulsification of M812/T85 mixtures. A study of the effect of water added to the original 70%M812/30%T85 mixture on subsequent self-emulsification showed that emulsification properties were unaffected by up to 5%^{w}/w water solubilized in the oil/surfactant mixture. At 6.49% water the mixture formed the liquid crystalline phase described above. The latter phase resulted in fine emulsions but further addition of water to the original mixture subsequently resulted in

poor emulsification. Therefore emulsification may have taken place by initial solubilization of water in the oil/surfactant phase forming cylinders of liquid crystal locally at the interface. These cylindrical micelles may have allowed penetration of water through their hydrophilic cores resulting in extensive interfacial disruption.

The presence of electrolytes in the aqueous phase tended to reduce the efficiency of self-emulsification. Concentrations of HCl, NaCl and $CaCl_2$ below 10^{-4}Molar had no detectable effects on emulsification but as the concentration of electrolyte was increased to 10^{-3}M the emulsification times became longer and mean particle sizes were enlarged. Further increase in electrolyte concentration to 10^{-1}M resulted in poor emulsions with wide size ranges. The effect of HCl was generally stronger than that of the salts. Examination of phase behaviour of M812/T85/ 10^{-1}M HCl mixtures showed that, whilst the CST remained constant, the formation of liquid crystalline phases was suppressed by the presence of the electrolyte. This was probably due to an effect on the spacial arrangement of the oxyethylene chains of the surfactant. Moreover it was further evidence that the formation of the liquid crystalline phase may have been necessary to achieve self-emulsification.

The inclusion of benzoic acid (1-5%^{w}/w) in the original 70%M812/30%T85 mixture improved the efficiency of emulsification at 25°C. Phase studies showed that CST had been lowered by the presence of benzoic acid to approximately 30°C. In addition the regions of water solubilization and liquid crystal close to the CST were enlarged. Thus emulsification at 25°C was enhanced. This enhancement was great enough to overcome the deleterious effects of 10^{-1}M HCl disolved in the aqueous phase. Therefore the presence of 5% benzoic acid would have aided dispersion in the stomach contents. It was clear from the studies described above that the effects of dissolved substances on emulsification of oil/surfactant mixtures may be important and that the effects may be predicted by studies of phase behaviour at equilibrium.

The nature of the oil component was as important to self-emulsification as the nature of the surfactant material. Polar oils such as octanol or fatty acids did not form self-emulsifying mixtures with T85 at ambient temperatures. Polar oil/T85 mixtures tended to form water-in-oil emulsions. They were comparable to M812/T85 mixtures containing less than 15%^{w}/wT85. Studies of phase behaviour of polar oil/T85/water mixtures at equilibrium corresponded to poor emulsification properties. Mixtures of octane/T85 and glyceryl trioleate/T85 were also poorly emulsifying. The properties of these mixtures were interesting because certain octane/T85 and glyceryl trioleate/T85 mixtures had critical solubilization regions at ambient temperatures.

However even those mixtures with oil/T85/water CSTs close to 25°C were poorly emulsifying at 25°C. Inspection of the phase behaviour showed that no liquid crystalline phases were formed close to the critical solubilization region for octane/T85/water mixtures at equilibrium. Glyceryl trioleate/T85/water mixtures formed some liquid crystalline material but this was of the lamellar type; comparable to that formed by M812/T85 mixtures containing more thatn 50%T85 at 25°C. These results further indicated the significance of the liquid crystals formed by Type B M812/T85 mixtures. Examination of the microstructure of the latter liquid crystalline phase is an important object for further study.

During a study of alternative surfactant materials it was found that M812/T80 mixtures self-emulsified slowly when the concentration of T80 was greater than 50%^{w}/w. The particle size of self-emulsified M812/T80 mixtures decreased as the proportion of T80 in the original mixture increased. These properties were explained by the hydrophilic nature of T80. The self-emulsification of M812/T80 mixtures was thought to be due to diffusion of T80 into the aqueous phase. Indeed the pure surfactant dissolved slowly in water to form a micellar solution. Addition of S85, a lipophilic surfactant, did not aid (but generally hindered) the emulsification of M812/T85 mixtures. Therefore the assumption that the use of 'mixed surfactants' <u>per se</u> should enhance self-emulsification is incorrect.

In summary the current study indicated that self-emulsifying oil/surfactant mixtures could be formulated by reference to studies of phase behaviour at equilibrium. Efficient emulsification was achieved by adjusting formulations such that:

1. Progressive addition of water to the oil/surfactant mixture (I_o phase) close to the critical solubilization region resulted in solubilization of water until the I_o phase was saturated followed immediately by a transition to a single liquid crystalline phase.

2. The CST of the oil/surfactant/water mixture was a little above the temperature at which emulsification was to take place.

The temperature during the initial emulsification process was crucial to the quality of the final emulsion. Once self-emulsification had taken place the particle size of the emulsion was comparatively unaffected by heating or cooling the final emulsion.

Estimation of biological absorption rates after oral administration of benzoic acid did not detect statistically significant differences between absorption of benzoic acid from various formulations. Benzoic acid was absorbed quickly and, largely, completely from all formulations which may suggest that absorption of 'well-absorbed' drugs would not be affected by formulation in a self-emulsifying vehicle. However the scale of the current study was not large enough to reach a firm conclusion. An extension of

the study using additional subjects would be interesting. Future biological work should also include a study of the extent of absorption of poorly absorbed drugs.

APPENDICES

APPENDIX ONE

DESCRIPTION OF THE APPARATUS USED FOR COMPARISON OF RATE OF SELF-EMULSIFICATION

Figure A.1.1 - A.1.4 represent the construction of the apparatus used for preparation of self-emulsified systems.

A glass cell sat within a nephelometric device mounted in a wooden housing. The housing was raised by means of a laboratory jack to meet a glass paddle and micrometer syringe which were held by clamps to a metal frame. The frame, in turn, was bolted into a wooden base which also held the laboratory jack in a fixed position.

Incident light was provided by a 55W projector lamp via a flexible fibroptic light guide which was able to move with the nephelometer housing. The glass paddle was rotated at constant speed ($\pm$ 1%) by an electric motor (Citenco KQPS.22) stabilised at low speed using a thyristor-controlled variable resistance (Trager Ltd.). Emulsifiable material was injected by volume into excess aqueous phase (15ml) using an Agla micrometer syringe (Wellcome Res. Ltd.) fitted with disposable needles (Sabre size 25G x 15/16", Gillette Surgical Ltd.).

Figure A.1.2 represents the nephelometer and cell in vertical cross-section. Scattered light was gathered by fibroptic light guides mounted in the nephelometer block as shown in Figure A.1.2 (and in horizontal cross-section in Figure A.1.3). The intensity of light was

Figure A.1.1 Construction of the apparatus used for self-emulsification and comparison of emulsification rate.

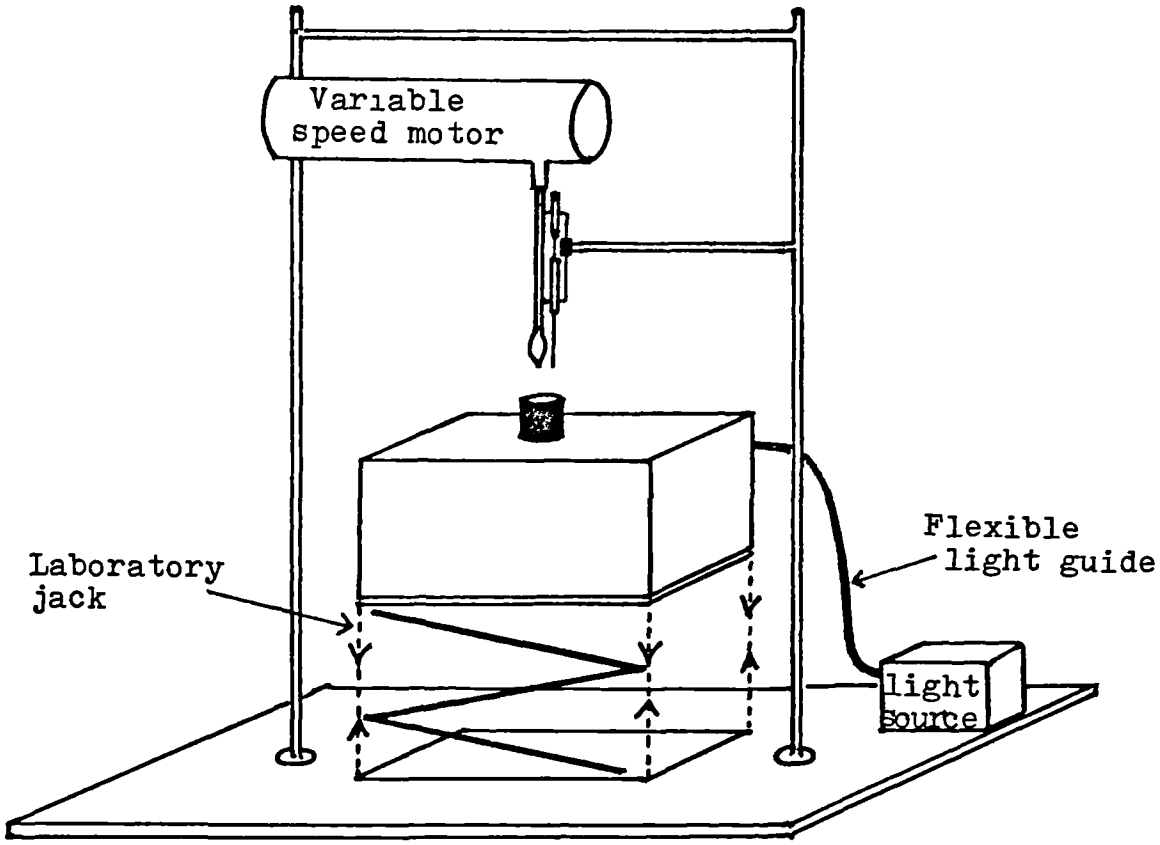


Figure A.1.2 Cell and nephelometer in vertical cross-section.

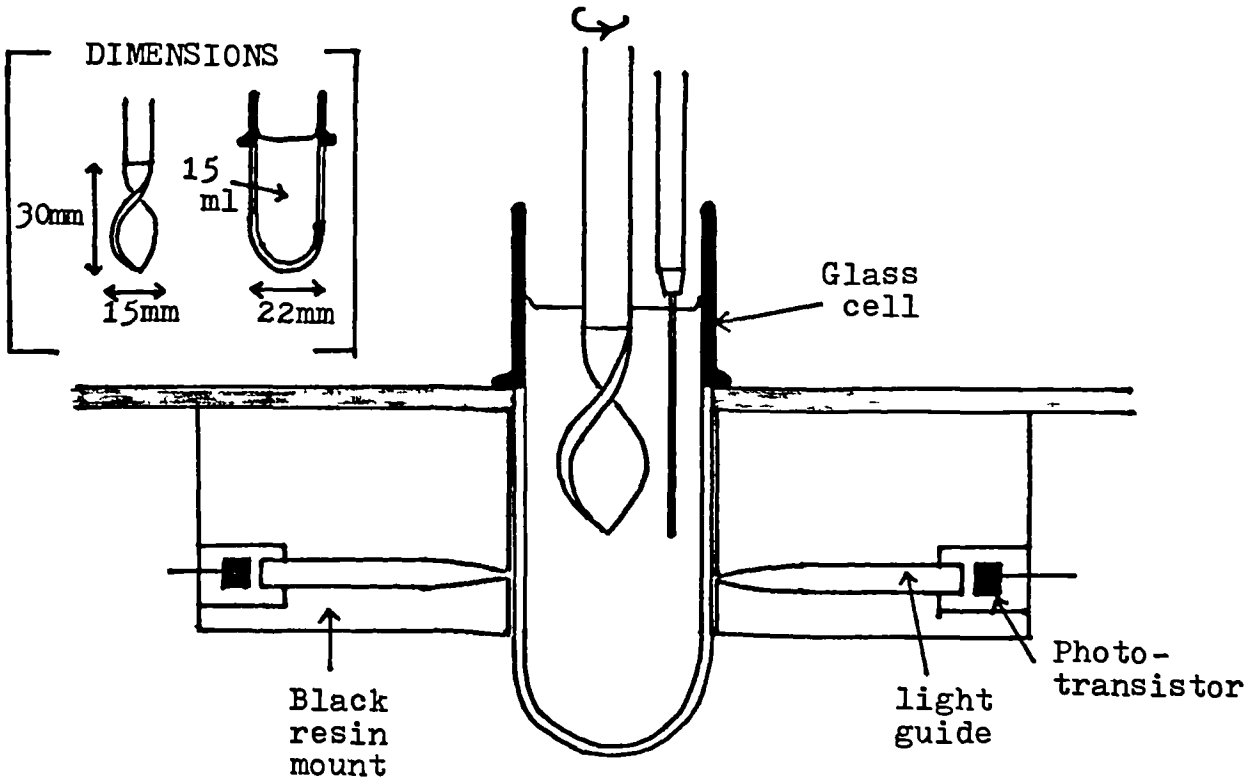


Figure A.1.3 Nephelometer in horizontal cross-section

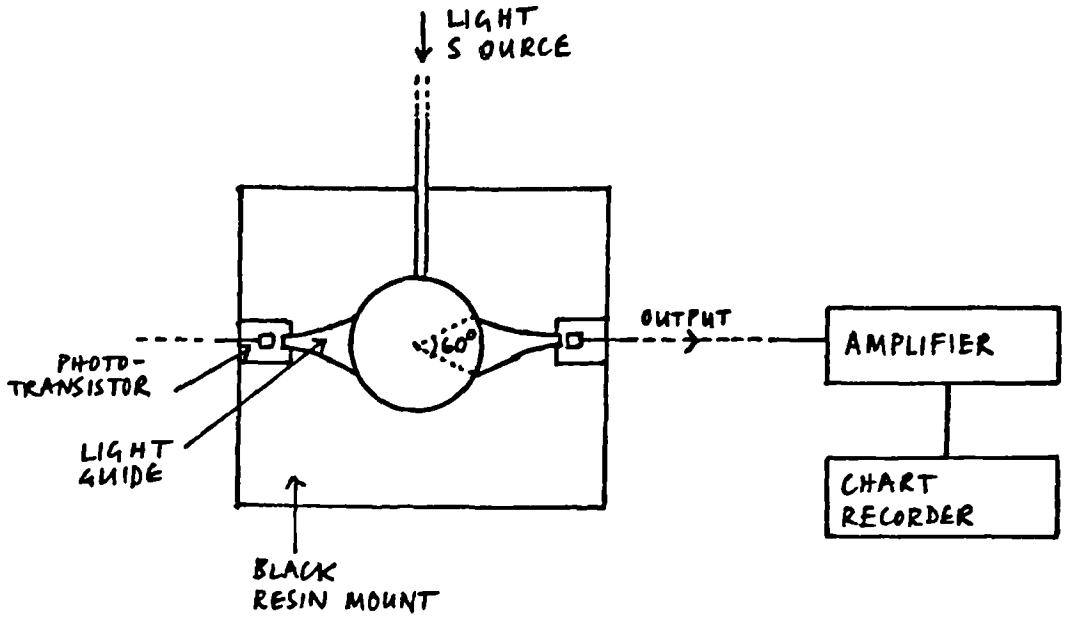
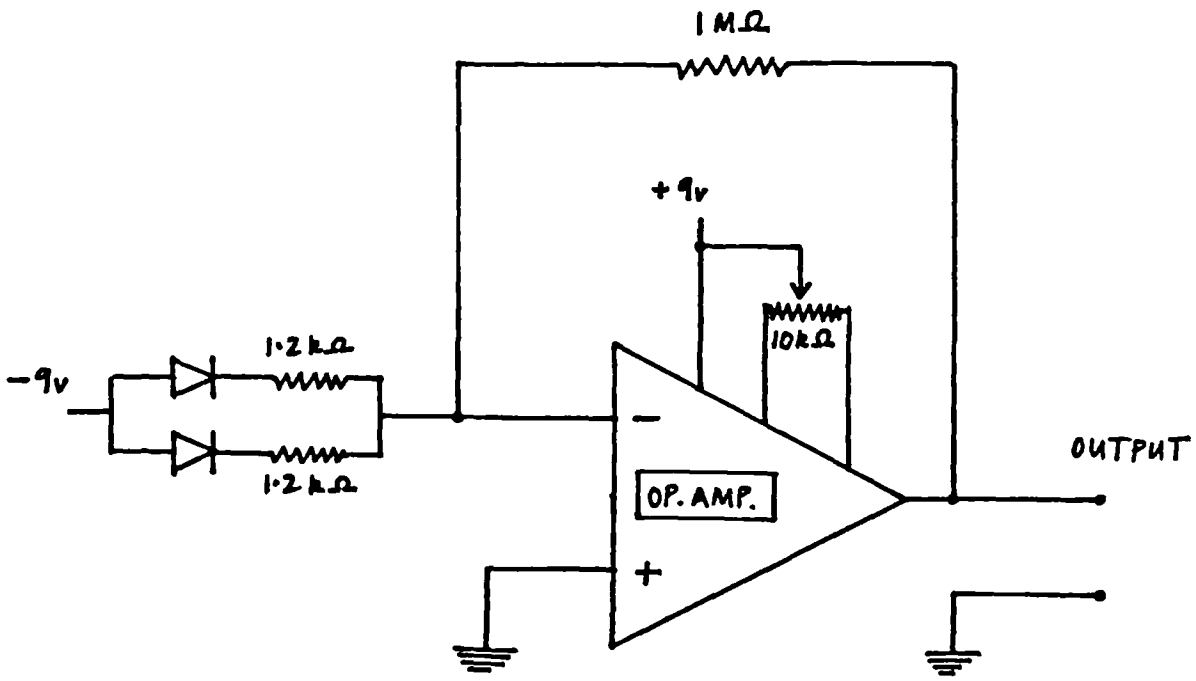


Figure A.1.4 Schematic diagram of the amplifier circuit



measured using a pair of phototransistors amplified by the circuit represented schematically by Figure A.1.4. The output from the amplifier was plotted against time using a chart recorder.

The value of intensity of scattering for a dispersed system (D_s), first described by Rayleigh[204], depends on a wide variety of factors; particle number per unit volume (N), particle size (expressed as volume - v), the wavelength of incident light (λ), the refractive indices of the two materials (n_o, n_1) and the angle of measurement (θ).

$$D_s = \frac{9.\pi^2.N.v^2}{2.d^2.\lambda^4} \cdot \frac{(n_1^2 - n_o^2)}{(n_1^2 + 2n_o^2)} \cdot (1 + \cos^2\theta) \cdot D_o \quad .....(A.1)$$

The nephelometer currently described gathers scattered light from many angles and represents the value for a sum of several angles of measurement. However for any set of measurements using the same instrument, similar conditions and materials many of the terms in the above equation are constant. The equation can be reduced to the form

$$D_s = j.N.v^2 \quad .....(A.2)$$

where j is a 'machine constant'.

It was of interest to test the properties of the nephelometer using a series of polystyrene latices of narrow size range. If Rayleigh's equation held then one would expect a plot of $\delta D_s / \delta N$ against v^2 to be linear. Infact Rayleigh's work predicts that ideality would only occur if the particles were significantly smaller than the wavelength of the incident light (radius $< \lambda/20$). Therefore over the range 0.1 to 1 μm latex diameter one would expect non-ideal behaviour. Figure A.1.5 shows a plot of relative $\delta D_s / \delta N$ against v^2. Unexpectedly the plot fits equation A.2 up to latex diameters of about 0.6 μm. Similar behaviour has been reported for light scattering of latices in a laser beam[38]. The curve deviates from linearity as particle diameter approaches 1.0 μm when each particle began to act as a multiple scattering centre. In Table A.1.1 the slope $\frac{\delta Ds}{\delta C}$ is also included. This represents the slope of the plot of relative D_s versus $\%^w/w$ concentration. This parameter indicates the significance of particle size if a fixed quantity of material could emulsify to various precise particle sizes. Figure A.1.6 shows that emulsions formed with mean sizes between 0.4 and 0.8 μm would be expected to scatter light strongly. Low intensities of scattering would be characteristic of very fine and coarse emulsions. The particle sizes which scatter light strongly depend on the wavelength of the incident light. For a given concentration of dispersed phase, by weight, strong light scattering was typical of particle sizes in the region of the wavelength of the incident light.

Figure A.1.5 Slope $\delta Ds/\delta N$ versus latex volume^2 (v^2)

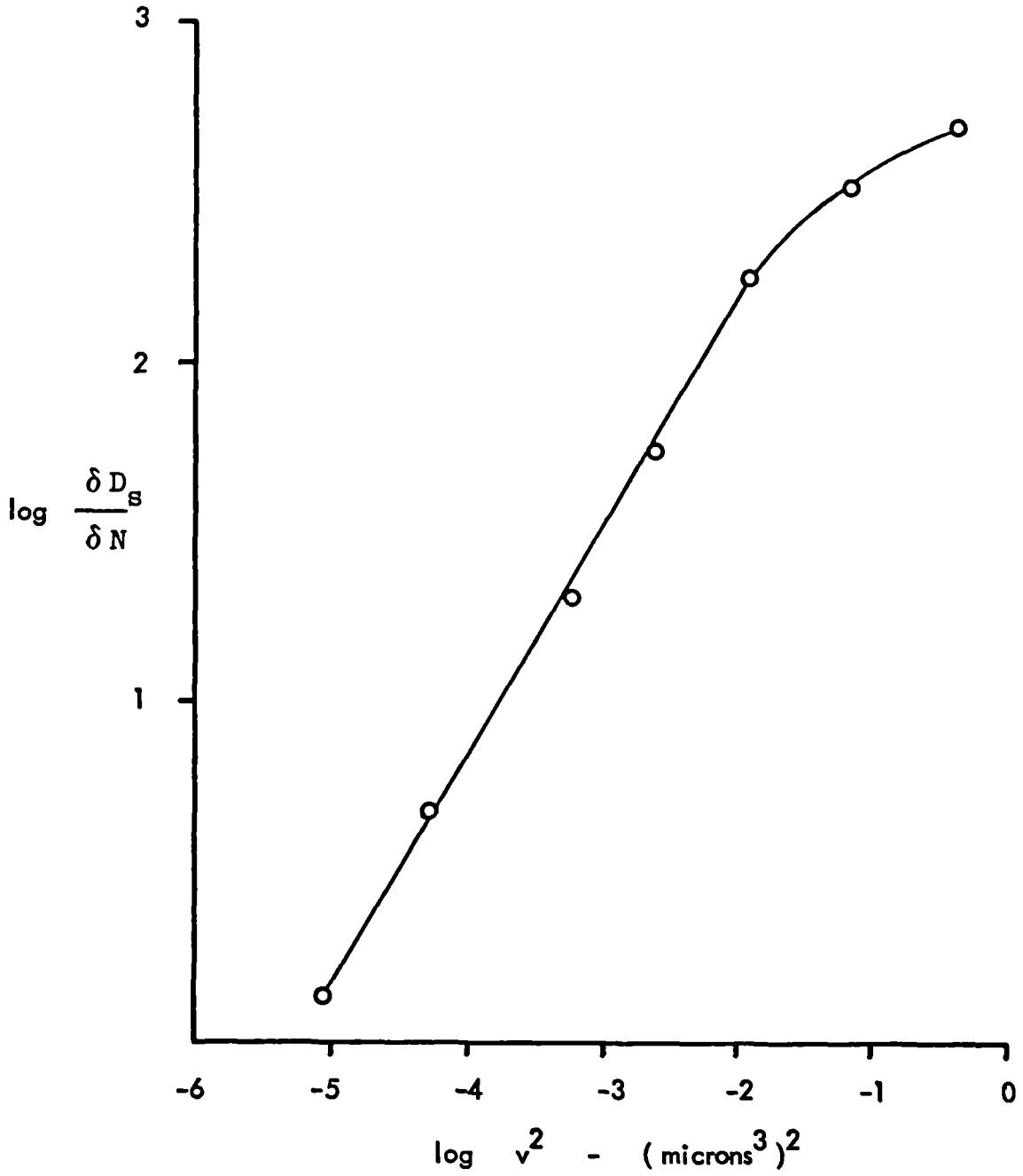
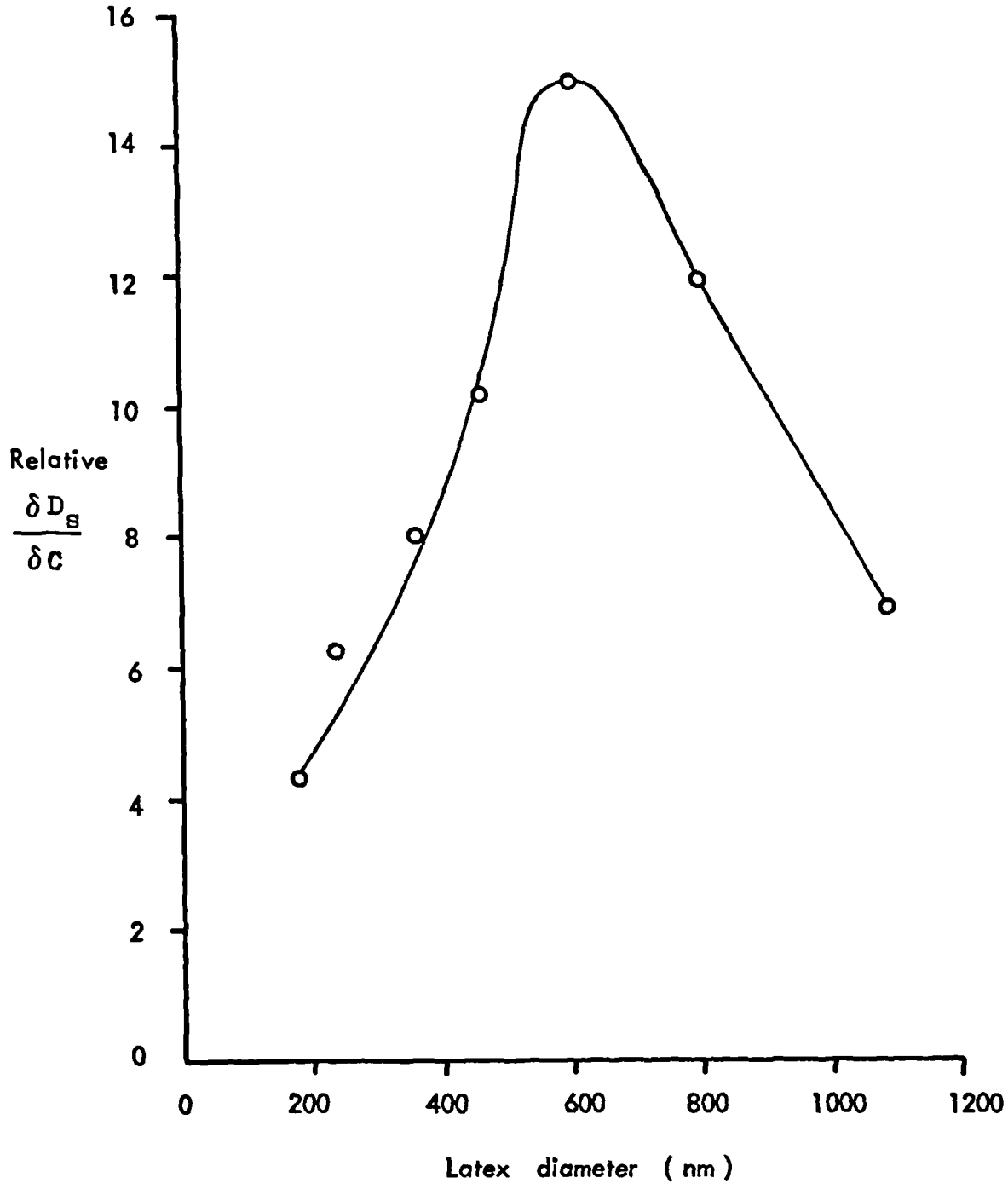


Table A.1.1 Relative light scattering of polystyrene latex dispersions.

Particle diameter (microns)	Particle volume^2 (v^2) (microns^3)^2 $\times 10^{-6}$	Slope relative $\frac{\delta D_s}{\delta N}$	Slope relative $\frac{\delta D_s}{\delta C}$	correlation coefficient
0.18	9	1.4	4.27	0.9982
0.24	52	4.8	6.25	0.9990
0.36	597	20.0	7.98	0.9984
0.46	2597	54.3	10.16	0.9992
0.60	12790	177.7	14.96	0.9997
0.80	71870	335.2	11.94	0.9997
1.09	459800	492.7	6.92	0.9999

Figure A.1.6 Slope Rel($\delta D_s / \delta C$) versus latex diameter

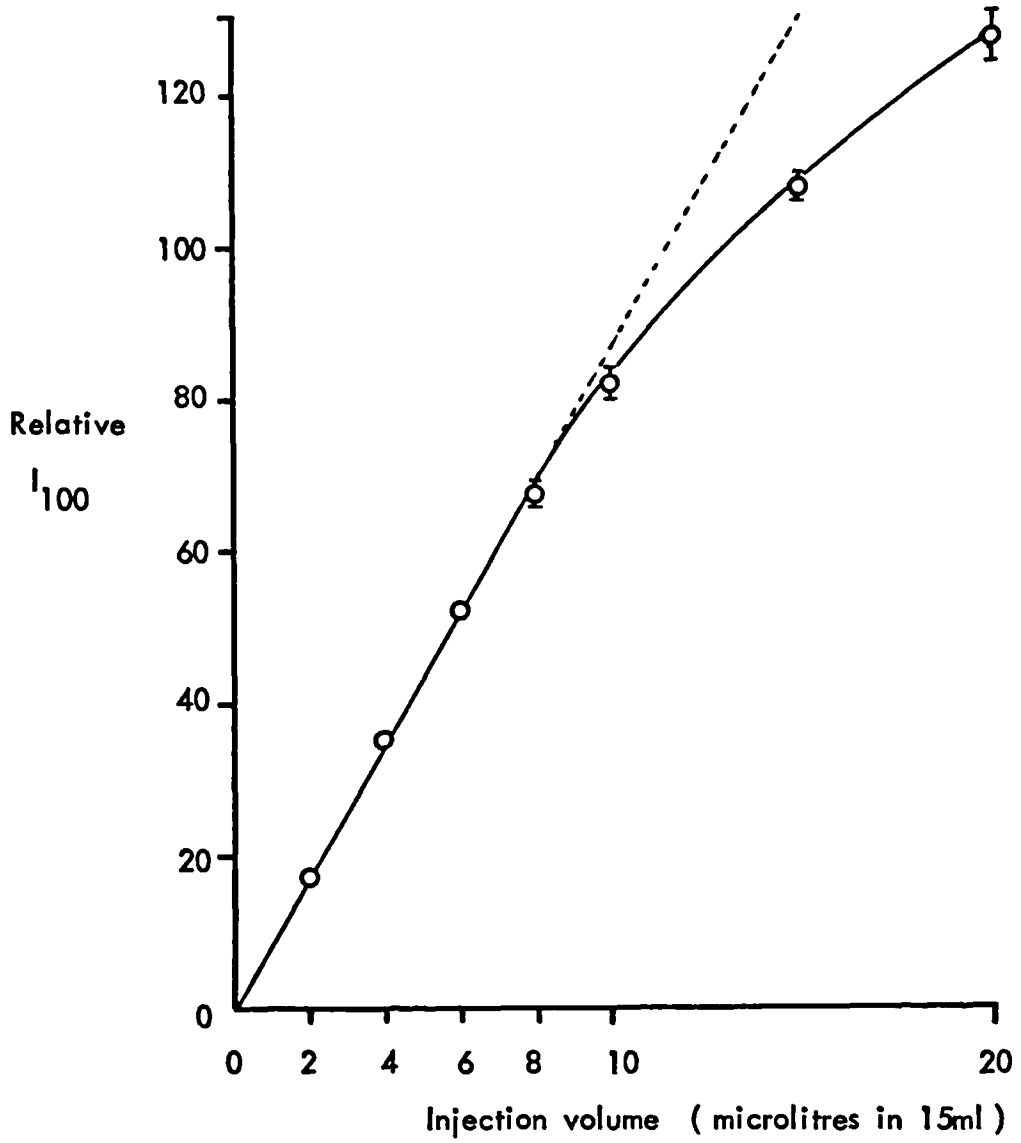


The light scattering properties of polydisperse emulsion systems are therefore complicated by the wide range of particle sizes present. Differences in intensity of scattered light can not be attributed to any particular property of each emulsion. However for each emulsion the intensity of scattering was proportional to emulsion concentration over the working range. The volume of emulsifiable material injected into the cell was generally 10μl . At higher concentration a plot of intensity against concentration began to deviate from linearity (Figure A.1.7)

The mixing efficiency of the paddle was tested using a preformed emulsion (Intralipid 20% - Kabi Vitrum Ltd.). At rotor speeds greater or equal to 100 rpm, homogeneity was achieved within four seconds, timed from the point of emulsion injection. These conditions were considered satisfactory.

The sensitivity of the scattering intensity measurement was adjusted to suit efficient self-emulsifying mixtures (50mv full-scale deflection). Using this amplification, relative intensities (Rel I_{100} values - see Fig. 3.1) for efficiently emulsifying mixtures were in the region 50-100 units. The size of I_{100} was an indication of the state of dispersion; poor emulsions had Rel I_{100} values of less than 20.

Figure A.1.7 Effect of injection volume on Rel I_{100} (70%M812/30%T85 in distilled water at 25°C)



Macroscopically, self-emulsification appeared to occur by erosion of fine droplets from the bulk material. It was not surprising therefore that the Intensity versus time curves had a similar appearance to dissolution curves (Fig. 3.1). Simple mathematical models have been used to describe dissolution curves[205-206] (although indeed their value has been questioned[207]). It was tempting to use such a model to express self-emulsification curves. However as the value of Rel I was not a direct measure of the number of particles present or their particle sizes, mathematical use of the value Rel I was considered inappropriate and rate of self-emulsification was compared using $t_{I\%}$ values (see Chapter 3).

APPENDIX TWO

DEVELOPMENT OF AN ASSAY METHOD FOR BIOPHARMACEUTICAL EXPERIMENTS

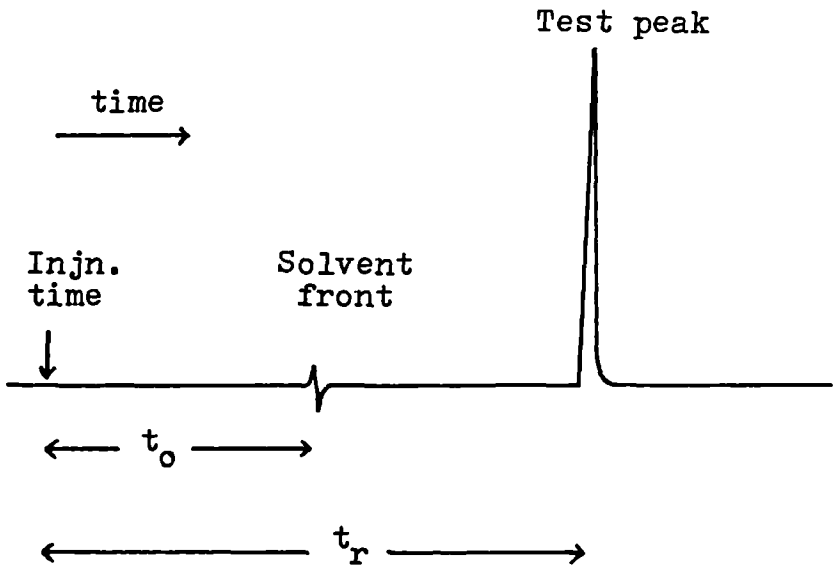
Several assay techniques for determination of benzoic and hippuric acids in urine have been described by previous authors. Quick[43] and Hirsheimer [44] used gravimetric methods; Amsel and Levy[46] a photometric technique after extraction of the urine with organic solvents. Wu and Elliot[45] extracted the urine using an ion-exchange resin and then used a similar photometric technique. In 1973 Langenbeck and Seegmiller[208] reviewed assay methods for hippuric acid and concluded after an exhaustive study that the preferred technique utilised gas-liquid chromatography (G.L.C.) after extraction using the method of Dalgleish et al[209]. Derivitisation was necessary for resolution by G.L.C.

All the assay techniques described above are laborious and involve urine extraction and/or chemical reaction prior to determination. For these reasons it was decided to attempt to develop an assay of the urine using high-performance liquid chromatography (H.P.L.C.) which would be rapid in comparison with established methods and possibly more precise.

A non-polar material consisting of long chain (C_{18}) aliphatic groups siloxane bonded onto silaceous support particles of mean size 10 μm (Partisil PXS-ODS) was selected as the stationary phase. Materials partition

between this non-polar phase and a moving phase. Such stationary phases are designed for use with polar eluents such as methanol and water. Materials were detected using a Cecil CE212 ultraviolet spectrophotometer. The absorption maxima for hippuric and benzoic acids were coincident at a wavelength of 230nm. Absorption was sufficient to allow the use of wavelengths up to 250nm.

Separation of benzoic and hippuric acids was not achieved using mixtures of water and methanol as the moving phase. On addition of acetic acid to the eluent separation was achieved. Table A.2.1 represents the effect of eluent composition on retention of hippuric and benzoic acids. Retention is expressed as sample capacity as explained below.



Sample capacity factor, $k' = \frac{t_r - t_o}{t_o}$

Table A.2.1 Effect of moving phase composition on retention of hippuric and benzoic acids stationary phase : Partisil PXS 25 ODS

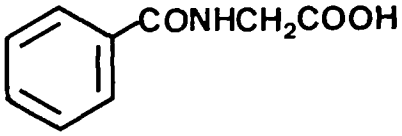
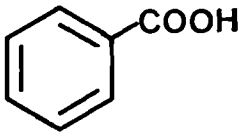
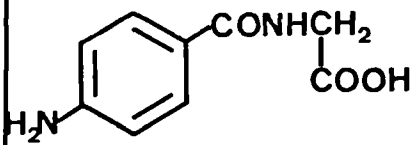
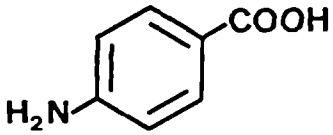
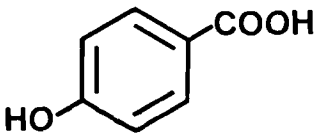
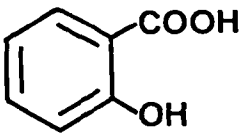
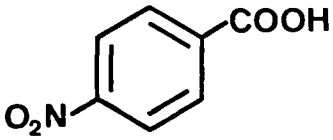
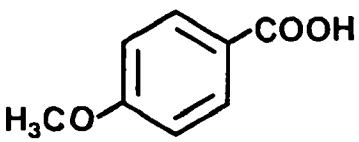
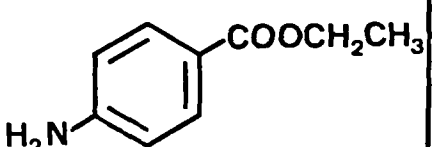
MOVING PHASE COMPOSITION %v/v			SAMPLE CAPACITY FACTOR (k')		COMMENTS ON RESOLUTION
Methanol	Water	Acetic acid	Hippuric acid	Benzoic acid	
10	90	-	0.17	0.17	peaks coincident
50	50	-	0.08	0.17	peaks overlapped
80	20	-	0.00	0.08	peaks overlapped considerably
10	87.5	2.5	1.25	2.83	peaks resolved
25	72.5	2.5	0.67	1.83	peaks resolved
40	57.5	2.5	0.50	1.30	peaks just resolved
50	47.5	2.5	0.42	0.5	peaks overlapped

An internal standard was required with which to compare test peaks. A series of benzoates were screened for this purpose. The benzoates were all eluted more rapidly as methanol concentration in the moving phase was increased. Inclusion of acetic acid in the moving phase had more complex effects but generally helped to reduce tailing as well as controlling resolution. Table A.2.2 compares sample capacities for the series of benzoates using methanol/water/acetic acid (40/57.5/2.5) as the eluent.

Subsequently an analysis of urinary hippuric acid was attempted using anisic acid as an internal standard. The method was unsatisfactory for direct urine analysis using the above eluent because the ultraviolet absorption of other urinary constituents interfered with the hippuric acid peak. The extraction method of Dalgleish et al[209] 'cleaned' the urine sufficiently for analysis but was undesirable. It was decided that time would be wisely spent developing a direct urine assay.

Hippuric acid was separated from other urinary constituents by reducing the methanol and acetic acid contents of the eluent. This resulted in longer retention times for all benzoates. Having resolved hippuric acid from other urinary compounds the internal standard required reconsideration. Anisic acid was retained on the column too long and 'tailed' badly. This problem was solved by selecting p-OH benzoic acid as an alternative standard.

Table A.2.2 Retention study of a series of benzoates. moving phase : methanol/water/ acetic acid (40/57.5/2.5) staionary phase : Partisil PXS 25 ODS

CHEMICAL APPROVED NAME	CHEMICAL FORMULA	SAMPLE CAPACITY FACTOR (k')
hippuric acid		0.36
benzoic acid		1.16
p-amino-hippuric acid		0.11
p-amino-benzoic acid		0.33
p-hydroxy-benzoic acid		0.58
salicylic acid		1.33
p-nitro-benzoic acid		1.58
anisic acid		1.75
benzocaine		1.87

p-OH benzoic acid eluted between hippuric and benzoic acids and was resolved from the hippuric acid peak by a further modification of the eluent composition. Table A.2.3 describes the effect of eluent composition on the resolution of hippuric, p-OH benzoic and benzoic acids. The optimum eluent was methanol/water/acetic acid (10/89.95/0.25). The significant reduction in acetic acid content had the advantage of allowing the use of spectrophotometer wavelengths below 240nm. Therefore the absorption maximum of 230nm was used.

Table 4.2.4 represents the calibration of the assay in distilled water. Table A.2.5 lists the results of 'spiking' experiments in a sample of normal urine. The assay was very precise. Standard deviations between replicate injections were less (often considerably less) than 2% of the means. Determination of the two compounds in urine suggested that the assay was sufficiently precise as to require only one determination for each urine sample assayed.

Table A.2.3 Effect of moving phase composition on retention of hippuric, p-OH benzoic and benzoic acids.
Stationary phase : Partisil PXS 25 ODS

MOVING PHASE COMPOSITION %v/v			SAMPLE CAPACITY FACTOR $k' = (t_r - t_o) / t_o$			COMMENTS ON RESOLUTION
methanol	water	acetic acid	hippuric acid	p-OH benz acid	benzoic acid	
10	87.5	2.5	1.60	2.20	4.60	hipp./p-OH-benz. overlap
5	92.5	2.5	2.00	2.88	4.80	hipp./p-OH-benz. overlap
10	85	5	1.36	1.52	3.56	hipp./p-OH-benz. overlap
20	79	1	1.20	1.72	3.48	hipp./p-OH-benz. overlap other urinary compounds/hipp
10	89.5	0.5	2.20	2.80	5.00	hipp./p-OH-benz. overlap slightly
10	89.75	0.25	2.80	4.08	6.80	all peaks resolved from each other and common urinary constituents.

Table A.2.4 Calibration of assay for hippuric and benzoic acids in water using p-OH benzoic acid as a standard.
Stationary phase : Partisil PXS 25 ODS
moving phase : methanol/water/acetic acid (10:89.75:0.25)

CONCENTRATION OF MATERIAL IN INJECTION (mg/ml)			PEAK HEIGHT RATIO (mean and standard deviation)	
hippuric acid	p-OH-benz. acid	benzoic acid	hippuric / p-OH-benz.	benzoic / p-OH-benz.
0.2	0.3	0.2	1.237 0.002	1.110 0.003
0.15	0.3	0.15	0.927 0.003	0.815 0.006
0.1	0.3	0.1	0.624 0.001	0.554 0.003
0.075	0.3	0.075	0.476 0.009	0.417 0.009
0.05	0.3	0.05	0.322 0.001	0.286 0.001
0.025	0.3	0.025	0.162 0.004	0.147 0.004

Linear Regression Analysis

Concentration (x - axis) versus mean peak height ratio (y - axis).

	hippuric acid	benzoic acid
slope	6.113	5.459
correlation coefficient	0.9999	0.9998

Table A.2.5 Assay for hippuric and benzoic acids in urine ('spiked urine' test).

Concentration of hippuric acid in 'blank' urine sample = 0.0435 mg/ml.

CONCENTRATION OF DRUG ADDED TO URINE (mg/ml)			HIPPURIC ACID			BENZOIC ACID	
hippuric acid	p-OH-benz acid	benz. acid	concn. detected (mg/ml)	concn. minus blank (mg/ml)	determd. value as % of 'spike'	concn. detected (mg/ml)	determd. value as % of 'spike'
0.05	0.3	0.05	0.0930	0.0495	99.0	0.0498	99.6
0.075	0.3	0.075	0.1175	0.0740	98.7	0.0748	99.8
0.1	0.3	0.1	0.1414	0.0979	97.8	0.0992	99.2
0.15	0.3	0.15	0.1897	0.1462	97.5	0.1476	98.4
0.2	0.3	0.2	0.2418	0.1983	99.2	0.1979	99.0

REFERENCES

1. Wagner, J.G.,
"Biopharmaceutics and Recent Pharmacokinetics", p.65,
Drug Intelligence Publications, (1971).

2. Gibaldi, M. and Feldman, S.,
J.Pharm. Sci., 59, 579, (1970).

3. Bates, T.R. and Gibaldi, M.,
"Current concepts: Biopharmaceutics",
Lea and Febiger, Ed. Swarbrick, J., (1970).

4. Gibaldi,M .,
"Introduction to Biopharmaceutics",
Lea and Febiger, (1970).

5. Wagner, J.G.,
"Biopharmaceutics and Recent Pharmacokinetics", p.98,
Drug Intelligence Publications, (1971).

6. Nelson, E.,
Clin. Pharm. Therap., 3, 673, (1962).

7. Kaplan, S.A.,
Drug Metab. Rev., 1, 15, (1972).

8. Greco, G.A., Moss, E.L. and Foley, E.J.,
Antibiot. A., 7, 663, (1959).

9. Crounse, R.G.,
J. Invest. Dermat., 37, 529, (1961).

10. Kraml, M., Dubue, J. and Beall, D.,
Can. J. Biochem. Physiol., 40, 1449, (1962).

11. Kabasakalian, P. et al,
J. Pharm. Sci., 59, 595, (1970).

12. Carrigan, P. and Bates, T.R.,
J. Pharm. Sci., 62, 1476, (1973).

13. Bates, T.R. and Sequeira, J.A.,
J. Pharm. Sci., 64, 793, (1975).

14. Bates, T.R. and Carrigan, P.J.,
J. Pharm. Sci., 64, 1475, (1975).

15. Chakrabarti, S. and Belpaire, F.M.,
J. Pharm. Pharmac. 30, 330, (1978).

16. Thoma, K., Pfaff, G. and Quiring, K.,
J. Pharm. Pharmac., 30, 270, (1978).

17. Feinstone, W.H., Wolff, R. and Williams, R.D.,
J. Bacteriol., 39, 47, (1940).

18. Lewis, J.M., Cohlar, S.Q. and Messina, A., Paediatrics, 5, 425, (1950).

19. Daeschner, C.W. et al, Am. Med. Assoc. J. Disease in Children, 93, 370, (1957).

20. Wagner, J.G., Gerrard, E.S. and Kaiser, D.G., Clin. Pharmac. Therap., 7, 610, (1966).

21. Kakemi, K. et al, Chem. Pharm. Bull., 20, 708, (1972).

22. Minkov, E. et al, Pharm. Ind., 36, 446, (1974).

23. Gibaldi, M., Fed. Proc., 29, 1343, (1970).

24. Gibaldi, M. and Feldman, S., J. Pharm. Sci., 59, 579, (1970).

25. Elworthy, P.H., Florence, A.T. and Macfarlane, C.B., "Solubilization by Surface-active Agents", p.248, Chapman and Hall, (1968).

26. Feldman, S. and Reinhard, M.R., J. Pharm. Sci., 65, 1460, (1976).

27. Gullikson, G. et al, Gastroenterology, 73, 501, (1977).

28. Whitmore, D.A., Brookes, L.G. and Wheeler, K.P., J. Pharm. Pharmac., 31, 277, (1979).

29. Walters, K.A., Dugard, P.H. and Florence, A.T., J. Pharm. Pharmac., 33, 207, (1981).

30. Knox, M.T. and Malkinson, C.N., Rend. Gastroenterol, 3, 115, (1971).

31. Yamahira, Y. et al, J. Pharmacodyn., 1, 160, (1978).

32. Bloedow, D. and Hayton, W., J. Pharm. Sci., 65, 328, (1976).

33. Yamahira, Y. et al, Int. J. Pharmaceutics, 3, 23 (1979).

34. Davis, S.S., J. Clin. Pharmacy, 1, 11, (1976).

35. Engel, R.H. and Fahrenbach, M.J., Proc. Soc, Exp. Biol, Med., 129, 772, (1968).

36. Engel, R.H. Riggi, S.J. and Fahrenbach, M.J.,
Nature, 219, 856, (1968).

37. Engel, R.H. and Riggi, S.J.,
J. Pharm. Sci., 58, 1372, (1969).

38. Lee, G.W.J.,
Ph.D Thesis, University of London, (1980).

39. O'Neill, D.K. and Alexander, A.E.,
J. Sci. Food Agric., 16, 750, (1965).

40. Elworthy, P.H. and Treon, J.F.,
"Nonionic Surfactants", Chapter 28, Ed. Shick, M.J.,
Arnold, London, (1967).

41. Collaborative International Pesticides Analytical Council,
Handbook, 1, (1975).

42. Groves, M.J.,
Chem. and Ind., p.417, (1978).

43. Quick, A.J.,
J. Biol. Chem., 92, 65, (1931).

44. Hirsheimer, A.,
Am. J. Obst. Gynaec., 37, 363, (1939).

45. Wu, H. and Elliot, H.C. Jnr.,
J. App. Physiol., 16, 553, (1961).

46. Amsel, L.P. and Levy, G.,
J. Pharm. Sci., 58, 321 (1969).

47. Williams, R.T.,
"Detoxification Mechanisms", Chapter 11
Chapman and Hall, (1959).

48. Aveyard, R. and Vincent, B.,
Prog. Surface Science, 8, 59, (1977).

49. Becher, P.,
"Emulsions : Theory and Practice", 2nd Ed., Chapter 7,
Reinhold, (1966).

50. Gopal, E.S.R.,
"Emulsion Science", Chapter 1,
Ed. Sherman, P., Academic Press, (1968).

51. Bondy, C. and Sollner, K.,
Trans. Farad. Soc., 32, S56, (1936).

52. Rajagopal, E.S.,
Kolloid-Zeitschrift, 167, 17, (1959).

53. Krishnan, R.S., Venkatasubramanian, V.S. and Rajagopal, E.S.,
J. Colloid Sci., 16, 41, (1961).

54. Tsukiyama, S., Takamura, A. and Nakura, N.,
Chem. Pharm. Bull., 22, 1902, (1974).

55. Rumscheidt, F.D. and Mason, S.G.,
J. Colloid Sci., 17, 260, (1962).

56. Richardson, E.G.,
J. Colloid Sci., 5, 404 (1950).

57. Hayworth, C.B. and Treybal, R.E.,
Ind. and Eng. Chem., 42, 1174, (1950)

58. Carroll, B.J. and Lucassen, J.,
"Theory and Practice of Emulsion Technology",
Ed. Smith, A.L., Academic Press, (1976).

59. Rumscheidt, F.D. and Mason, S.G.,
J. Colloid Sci., 16, 238, (1961).

60. Hinze, J.O.,
A.I. Ch. E. JL., 1, 289, (1955).

61. Roger, W.A., Trice, V.G. and Rushton, J.H.,
Chem. Eng. Progress, 52, 515, (1956).

62. Vermeulen, T., Williams, G.M. and Langlois, G.E.,
Chem. Eng. Progress, 51, 85F, (1955).

63. Shinnar, R.,
J. Fluid Mech., 10, 259, (1961).

64. Sullivan, D.M. and Lindsay, E.E.,
Ind. and Eng. Chem. Fundamentals, 1, 87, (1962).

65. Landau , J. and Prochazka, J.,
Coll, Czech. Chem. Commun., 29, 1866, (1964).

66. Gad, J.,
Arch. Anat. Physiol, p.181, (1878).

67. Quincke, G.,
Weidenmans Ann., 35, 593, (1888).

68. Sternling, C.V. and Scriven, L.E.,
A.I. Ch. E. JL., 5, 514, (1959).

69. Gouda, J.H. and Joos, P.,
Chem. Eng. Sci., 30, 521, (1975).

70. Gurwitsch,
"Wissenschaftliche Grundlagen der Erdölbearbeitung", (1913),
Translated by Moore, Chapman and Hall, (1932).

71. Raschersky, N.,
Z. Physik, 46, 568 (1928).

72. McBain, J.W. and Woo, P.T.,
Proc. Royal Soc. A., 163, 182, (1933).

73. Neumann, H.J.,
Z Elektrochem., 66, 555, (1962).

74. Ruschak, K.J. and Miller, C.A.,
Ind. Eng. Chem. Fundamentals, 11, 534, (1972).

75. Kling, W. and Schwerttner, H.,
Melliand Textilber, 22, 21, (1941).

76. Kaminski, A. and McBain, J.W.,
Proc. Royal Soc., A 198, 447, (1949).

77. Hartung, H.A. and Rice, O.K.,
J. Colloid Sci., 10, 436, (1955).

78. Matalon, R.,
Trans. Farad. Soc., 36, 651, (1940).

79. Haydon, D.A.,
Nature, 176, 839, (1955).

80. Stackelburg, M.V., Klockner, E. and Mohrhaner, P.,
Kolloidzeitschrift, 115, 53, (1949).

81. Davies, J.T. and Haydon, D.A.,
Proc. 2nd Int. Congr. Surface Activity, p. 417,
Butterworths, (1957).

82. Davies, J.T. and Rideal, E.K.,
"Interfacial Phenomena", 2nd Ed.,
Academic Press, (1963).

83. Manfield, W.W.,
Australian J. Sci. Research, A5, 331, (1952).

84. Gilbert, E.D.,
Proc. 2nd Int. Congr. Surface Activity, p.447,
Butterworths, (1957).

85. Rehbinder, P.,
Proc. 2nd, Int. Congr. Surface Acrivity, p.476,
Butterworths, (1957).

86. Prince, L.M.,
J. Colloid Interface Sci., 23, 165, (1967).

87. Shah, D., Tamjeedi, A., Falco, J.W. and Walker, R.D. Jnr.,
A.I. Ch. E. JL., 18, 1116, (1972).

88. Subramanyan, S.V. and Gopal, E.S.R.,
Indian J. Technol, 5, 139, (1967).

89. Nikitina, S. and Mochalova, O.,
Kolloidnyi Zhurnal, 30, 264, (1968).

90. Ostrovskii, M.V., Baranbaum, R. Kh. and Abramzon, A.A.,
Kolloidnyi Zhurnal, 32, 565, (1970).

91. Wan, L. and Poon, P.,
Can. J. Pharm. Sci., 8, 98, (1973).

92. Millar, C. and Scriven, L.,
J. Colloid Interface Sci., 33, 360, (1970).

93. Rosano, H.L.,
J. Soc. Cosmet. Chem., 25, 609, (1974).

94. Reiss, H.,
J. Colloid Interface Sci., 53, 61, (1975).

95. Vincent, B. and Davis, S.S.,
Colloid Science : Specialist Periodical Reports, 3,
193, (1978).

96. Kislalioghi, S. and Friberg, S.,
"Theory and Practice of Emulsion Technology",
Ed. Smith, A.L., Academic Press, (1976).

97. Groves, M.J. and Mustafa, R.M.A.,
J. Pharm. Pharmac., 26, 671, (1974).

98. Groves, M.J. and de Galindez, D.A.,
Acta Pharm. Suecica, 13, 361, (1976).

99. Griffin, W.C.,
J. Soc. Cosmet. Chem., 1, 311, (1949).

100. Griffin, W.C.,
J. Soc. Cosmet. Chem., 5, 249, (1954).

101. Becher, P. and Griffin, W.C.,
"H.L.B.; a bibliography",
Atlas Chemical Industries Inc., (1970).

102. Mulley, B.A.,
"Nonionic surfactants" p 421, Ed. Schick, Marcel Dekker(1967)

103. Shinoda, K., Saito, H. and Arai, H.,
J. Colloid Interface Sci., 35, 624, (1971).

104. Schick, M.J.,
J. Phys. Chem., 68, 3585, (1964).

105. Schick, M.J.,
J. Colloid Sci., 18, 378, (1963).

106. Elworthy, P.H., Florence, A.T. and Macfarlane, C.B.,
"Solubilization by surface active agents", p.47,
Chapman and Hall, (1968).

107. Bailey, F.E. Jnr. and Collard, R.V.,
J. App. Polymer Sci., 1, 56, (1959).

108. Bailey, F.E. Jnr. and Koleske, J.V.,
"Nonionic surfactants", Chapter 23,
Ed. Schick, M.J., Marcel Dekker, (1967).

109. Friberg, S., Rydhag, L. and Doi, T.,
Adv. in Chemistry, 152, 28, (1976).

110. Rösch, M.,
"Nonionic Surfactants", Chapter 22,
Ed. Schick, M.J., Marcel Dekker, (1967).

111. Florence, A.T. and Rogers, J.A.,
J. Pharm. Pharmac., 24, 153, (1971).

112. Shinoda, K.,
"Solvent properties of surfactant solutions"
Ed. Shinoda, K., Marcel Dekker,(1967).

113. Ali, A.A. and Mulley, B.A.,
J. Pharm. Pharmac., 30, 205, (1978)

114. Shinoda, K. et al,
"Colloidal surfactants",
Academic Press, (1963).

115. Saito, H. and Shinoda, K.,
J. Colloid Interface Sci., 32, 649, (1970).

116. Arai, H.,
J. Colloid Interface Sci., 23, 348, (1967).

117. Shinoda, K. and Arai, H.,
J. Phys. Chem., 68, 3485, (1964).

118. Shinoda, K.,
J. Colloid Interface Sci., 24, 4, (1967).

119. Saito, H. and Shinoda, K.,
J. Colloid Interface Sci., 24, 10, (1967).

120. Shinoda, K. and Ogawa, T.,
J. Colloid Interface Sci., 24, 56, (1967).

121. Arai, H. and Shinoda, K.,
J. Colloid Interface Sci., 25, 396, (1967).

122. Shinoda, K. and Arai, H.,
J. Colloid Interface Sci., 25, 429, (1967).

123. Shinoda, K. and Saito, H.,
J. Colloid Interface Sci., 26, 70, (1968).

124. Kon-No, K. and Kitahara, A.,
J. Colloid Interface Sci., 34, 221, (1970).

125. Vincent, B.,
Colloid Science : specialist periodical reports,
1, 220, (1973).

126. Vincent, B.,
Colloid Science : specialist periodical reports,
2, 294, (1975).

127. Friberg, S., Lapczynska, I. and Gillberg, G.,
J. Colloid Interface Sci., 56, 19, (1976).

128. Friberg, S.,
J. Colloid Interface Sci., 66, 367, (1978).

129. Parkinson, C. and Sherman, P.,
J. Colloid Interface Sci., 41, 328, (1972).

130. Boyd, J., Parkinson, C. and Sherman, P.,
J. Colloid Interface Sci., 41, 359, (1972).

131. Shinoda, K. and Kuneida, H.,
J. Colloid Interface Sci., 42, 381, (1973).

132. Parkinson, C. and Sherman, P.,
Colloid and Polymer Sci., 255, 172, (1977).

133. Sherman, P. and Parkinson, C.,
Progr. Colloid and Polymer Sci., 63, 10, (1978).

134. Prince, L.M.,
J. Colloid Interface Sci., 23, 165, (1965).

135. Prince, L.M.,
J. Colloid Interface Sci., 29, 216, (1968).

136. Prince, L.M.,
J. Colloid Interface Sci., 31, 192, (1970).

137. Shinoda, K. and Friberg, S.,
Adv. Colloid Interface Sci., 4, 281, (1975).

138. Gillberg, G., Lechtinen, H. and Friberg, S.,
J. Colloid Interface Sci., 33, 40, (1970).

139. Shah, D.O. and Hamlin, R.,
Science, 171, 483, (1971).

140. Gerbacia, W. and Rosano, H.L.,
J. Colloid Interface Sci., 44, 242, (1974).

141. Ahmed, S.I., Shinoda, K. and Friberg, S.,
J. Colloid Interface Sci., 47, 32, (1974).

142. Rosano, H.L.,
J. Soc. Cosmet. Chem., 25, 609, (1974).

143. Prince, L.M.,
J. Colloid Interface Sci., 52, 182, (1975).

144. Sjöblom, E. and Friberg, S.,
J. Colloid Interface Sci., 67, 16, (1978).

145. Babayan, V.K.,
J. Am. Oil. Chem. Soc., 45, 23, (1968).

146. Technical Information,
Dynamit Nobel (UK) Ltd.

147. Merck Index, 9th Edition,
Merck and Co. Inc., (1976).

148. Technical Information,
Flourochem Ltd.

149. Benson, F.R.,
"Nonionic Surfactants", p.247,
Ed. Schick, M.J., Dekker, (1967).

150. Kubie, W.L., O'Donnell, J.L., Teeter, H.M. and Cowan, J.C.,
J. Am. Oil Chem. Soc., 40, 105, (1963).

151. Technical Datasheet,
Albright and Wilson Ltd.

152. Swarbrick, J.,
J. Soc. Cosmet. Chem., 19, 187, (1968).

153. Rosavear, F.B.,
J. Soc. Cosmet. Chem., 19, 581, (1968).

154. Boffey, B.J., Collison, R. and Lawrence, A.S.C.,
Trans. Farad. Soc., 55, 654, (1959).

155. Kratky, O., Leopold, H. and Stabinger, H.,
Technical Communication, Anton Paar K.G., Austria.

156. Lines, R.W. and Miller, B.V.,
Powder Technol, 24, 91 (1979).

157. Coulter Nanosizer TM Manual,
Coulter Electronics Ltd., (1980).

158. Elworthy, P.H. and Macfarlane, C.B.,
J. Chem. Soc.,Pt 1, 311, (1964).

159. Elworthy, P.H. and Mc.Donald, C.B.,
Kolloid Zeitschrift, 195, 16, (1964).

160. Schick, M.J.,
J. Am. Oil Chem. Soc., 40, 680, (1963).

161. Gray, V.R. and Alexander, A.E.,
J. Phys. Colloid. Chem., 53, 9, (1949).

162. Elworthy, P.H., Florence, A.T. and Macfarlane, C.B.,
"Solubilization by Surface Active Agents",
Chapman and Hall, (1968).

163. Schick, M.J.,
J. Phys. Chem., 67, 1796, (1963).

164. Lo, I. et al,
J. Colloid Interface Sci., 59, 319, (1977).

165. Winsor, P.A.,
"Solvent Properties of Amphiphilic Compounds",
Butterworths, (1954).

166. Ekwall, P., Mandell, L. and Fontell, K.,
J. Colloid Interface Sci., 19, 542, (1964).

167. Ekwall, P., Mandell, L. and Fontell, K.,
J. Colloid Interface Sci., 31, 530, (1969).

168. Ekwall, P., Mandell, L. and Fontell, K.,
Proc. 5th Int. Conf. Surface Activity, p.1059,
Barcelona, (1969).

169. Friberg, S. and Larsson, K.,
Adv. in Liquid Crystals, 2, 173, (1976).

170. Jurgen-Lohmann, L.,
Kolloid Zeitschrift, 124, 77, (1951).

171. McDonough, J.A., Tomme, W.J. and Holland, C.D.,
A.I.Ch. E. Jl., 6, 615, (1960).

172. Mustafa, R.M.A.,
Ph.D. Thesis, University of London, (1973).

173. Van der Waarden,M
J. Colloid Sci., 7, 140, (1952).

174. Ford, R.E.,
"Theory and Practice of Emulsion Technology",
Ed. Smith, A.L., Butterworths, (1976).

175. Schulman, J.H. and Rideal, J.K.,
Proc. Royal Soc., London, 122B, 46, (1937).

176. Schulman, J.H. and Cockbain, E.G.,
Trans. Farad. Soc., 36, 51, (1940).

177. Alexander, H.E. and Schulman, J.H.,
Trans. Farad. Soc., 36, 960, (1940).

178. Laurence, A.S.C.,
Nature, 170, 232, (1952)

179. Blakey, B.C. and Laurence, A.S.C.,
Dis. Farad. Soc., 18, 268, (1954).

180. Becher, P.,
"Emulsions: Theory and Practice", 2nd Ed., p.9,
American Chem. Soc., (1966).

181. Lin, T.J.,
J. Soc. Cosmet. Chem., 29, 117, (1978).

182. Matsumoto, S. and Sherman, P.,
J. Colloid Interface Sci., 33, 294, (1970).

183. Gibbons, R.A.,
Nature, 200, 665, (1963).

184. Becher, P.,
"Nonionic Surfactants", Chapter 15,
Ed. Schick, M.J., Marcel Dekker, (1967).

185. Ohba, N.,
Bull. Chem. Soc. Japan, 35, 1021, (1962).

186. Shinoda, K. and Arai, H.,
J. Colloid Sci., 20, 93, (1965).

187. Mulley, B.A. and Metcalf, A.D.,
J. Colloid Sci., 19, 501, (1964).

188. Friberg, S. and Mandell, L.,
J. Am. Oil Chem. Soc., 47, 149 (1970).

189. Becher, P.,
"Nonionic Surfactants", Chapter 18,
Ed. Schick, M.J., Marcel Dekker, (1967).

190. Kitchener, J.A. and Mussellwhite, P.R.,
"Emulsion Science", Chapter 2,
Ed. Sherman, P., Academic Press, (1968).

191. Becher, P., Tifilletti, S.E. and Machida, Y.,
"Theory and Practice of Emulsion Technology",
Ed. Smith, A.L., Academic Press, (1976).

192. Elworthy, P.H., Florence, A.T. and Rogers, J.A.,
J. Colloid Interface Sci., 35, 23, (1971).

193. Doscher, T.M., Myers, G.E. and Atkins, D.C. Jnr.,
J. Colloid Sci., 6, 223, (1951).

194. Maclay, W.N.,
J. Colloid Sci., 11, 272, (1956).

195. Kuriyama, K.,
Kolloid Zeitschrift, 181, 144, (1962).

196. Schick, M.J.,
J. Colloid Sci., 17, 801, (1962).

197. Nakagawa, T.,
"Nonionic Surfactants", Chapter 17,
Ed. Schick, M.J., Marcel Dekker, (1967).

198. Shinoda, K. and Takeda, H.,
J. Colloid Interface Sci., 32, 642, (1970).

199. Sunderland, V.B. and Enever, R.P.,
J. Pharm. Pharmac., 24, 804, (1972).

200. Florence, A.T., Madsen, F. and Puisieux, F.,
J. Pharm. Pharmac., 27, 385, (1975).

201. Donbrow, M. and Rhodes, C.T.,
J. Pharm. Pharmac., 18, 424, (1966).

202. Mukerjee, P.,
J. Pharm. Sci., 60, 1528, (1971).

203. Shinoda, K. and Sagitani, H.,
J. Colloid Interface Sci., 64, 68, (1978).

204. Rayleigh, Lord J.W.,
Phil. Mag., 44, 28, (1897).

205. Wagner, J.G.,
J. Pharm Sci., 58, 1253, (1969).

206. Langenbucher, F.,
J. Pharm. Pharmac., 24, 979, (1972).

207. Goldsmith, J.A., Randall, N. and Ross, S.D.,
J. Pharm. Pharmac., 30, 347, (1978).

208. Langenbeck, U. and Seegmiller, J.G.,
J. Chromatography, 80, 81, (1973).

209. Dalgleish, C.E. et al,
Biochem. J., 101, 792, (1966).